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Full Length Research Paper

Hypoglycemic activity of *Antidesma bunius* (L.) Spreng and *Mollugo oppositifolia* L. fresh and alcoholic extracts in the db/db diabetic mouse model

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***Antidesma bunius* (L.) Spreng and *Mollugo oppositifolia* L., also known as "bignay" and "malagoso" respectively, are commonly used medicinal plants in the Philippines to treat diabetes. However, the hypoglycemic effect of *A. bunius* (L.) Spreng and *M. oppositifolia* L. has not been reported to date. Therefore, this study was undertaken to investigate the hypoglycemic effects of the fresh and ethanolic extracts of the plants in db/db diabetic male mice. Fresh and ethanolic extracts (12.5 ml/kg and 250 mg/kg, respectively) were administered by gavage in db/db mouse, an animal model of diabetes. Blood from the tail snip was used to measure blood sugar levels on days 1 (D1), 8 (D8), 15 (D15), 22 (D22) and 29 (D29). Fresh extract of *M. oppositifolia* L. caused a significant reduction of blood glucose levels on D22 compared to untreated db/db mice ($P < 0.05$). On D29, fresh extract of *A. bunius* (L.) Spreng ($P < 0.01$), and fresh ($P < 0.01$) and ethanolic leaf extracts ($P < 0.05$) of *M. oppossitifolia* L. caused a significant reduction of blood glucose compared to untreated db/db mice. Fresh and ethanolic extracts of *A. bunius* (L.) Spreng and *M. oppositifolia* L. exhibited a significant hypoglycemic activity in db/db mice.**

Key words: *Antidesma bunius* L, Spreng, *Mollugo oppositifolia* L., diabetes, mouse, hypoglycemic.

INTRODUCTION

Diabetes mellitus is the most common chronic endocrine/ metabolic disease and considered as one of the 10 leading diseases in the Philippines afflicting one out of every five Filipinos (Philippine diabetes statistics

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(Villasenor and Lamadrid, 2006). A vast majority of Filipinos suffering from diabetes are afflicted with non-insulin dependent diabetes mellitus (Villasenor and Lamadrid, 2006). Characterized by elevated glucose levels that lead to metabolic abnormalities (Sun et al., 2008), diabetes mellitus in 2017 as reported by the International Diabetes Foundation has affected 425 million people globally (IDF Diabetes Atlas, 2017). The increase in prevalence of diabetes globally is projected in 2030 (Sarah et al., 2004), and that includes the Philippines.

Resulting from defects in insulin secretion, insulin action or both (Yki-Jarvinen, 1994), diabetes mellitus continues to pose a significant worldwide burden for the health care system. Associated with long term complications (Searls et al., 2012), diabetes mellitus is classified into two main types. Type 1 results from a deficiency of insulin and is conventionally treated with exogenous insulin, while type 2 results from insulin resistance or reduced insulin sensitivity and is usually treated with oral hypoglycemic agents (Rosak, 2002). Surveys on the use of medicinal plants for treating various diseases indicated a substantial number of respondents utilized herbal medicine (Hajdu and Hohmann, 2012). In the Philippines, despite the dearth of statistics on the efficacy of herbal medicines, complementary and alternative medicine is a traditional practice where diabetes mellitus is commonly treated using medicinal plants (Quisumbing, 1978; Reyes et al., 2017).

Antidesma bunius (L.) Spreng is a shrubby tree belonging to family Euphorbiaceae. Also known as “bignay” in the Philippines (Morton, 1987) and Mao Luang in English (Butkhup and Samappito, 2008). Ripe fruits of *A. bunius* (L.) Spreng contain different types of flavonoids, namely catechin, procyanidin B1 and procyanidin B2 (Butkhup and Samappito, 2008). *Mollugo oppositifolia* L. is a slender, spreading or ascending, smooth branched, annual herb belonging to family Molluginaceae (Quisumbing, 1978). Also known as “malagoso” in the Philippines, *M. oppositifolia* L. contains C-glycosyl-flavonoids (Chopin et al., 1984). Both herbal plants are popular in the Philippines as antidiabetic, however report on phytochemical screening (Doctor and Manuel, 2014) and validation of antihyperglycemic activity (Villasenor and Lamadrid, 2006) of a number of selected indigenous medicinal plants in the Philippines has not included *A. bunius* (L.) Spreng and *M. oppositifolia* L. In addition, *A. bunius* (L.) Spreng and *M. oppossitifolia* L are not included in the list of approved herbal medicine by the Department of Health in the Philippines.

Previous studies have shown that *A. bunius* (L.) Spreng has been used in traditional medicine for the treatment of various conditions (Udomkasemsab et al., 2018).

Although the hypoglycemic effects of *A. bunius* (L.) Spreng has been investigated in alloxan- (El-Tantawy et al., 2015) and streptozotocin- (Chowtivannakul et al., 2016) induced diabetic rat model, it has not been elucidated in db/db diabetic male mice. Some species of Molluginaceae has been demonstrated to exhibit a variety of activities including anti-inflammatory (Kim et al., 2008), antibacterial (Kim et al., 2008), spermicidal (Padma and Khosa, 1995), antifungal (Rajasekaran et al., 1993), immunomodulatory (Ferreira et al., 2003) and antioxidant (Lin et al., 2004). However, to our knowledge, the anti-diabetic potential of *M. oppositifolia* L. has not been elucidated. In the present study, the anti-diabetic potential of *A. bunius* (L.) Spreng and *M. oppositifolia* L. was investigated in an animal model of diabetes, the db/db mouse.

MATERIALS AND METHODS

Plant

Fruits and leaves of *A. bunius* (L.) Spreng and *M. oppositifolia* L. were collected in Pampanga State Agricultural University, Magalang, Pampanga, Philippines. Plant materials were identified and authenticated at the Botanical Herbarium, Museum of Natural History, University of the Philippines, Los Baños, College, Laguna, Philippines by Annalee S. Hadsall.

Animals

Three-month old db/db diabetic male mice weighing 37 to 44 g (n = 5/group) were used in this study. They were caged individually on a 12-h light schedule in a temperature controlled (20±2°C) colony room. They were given food and water *ad libitum*. The mice were treated in accordance with the guidelines set by the Institutional Animal Care and Use Committee of Pampanga Agricultural College. All efforts were made to utilize only the minimum number of animals necessary to produce reliable scientific data.

Preparation of fruit and leaf extracts

Fresh fruits of *A. bunius* (L.) Spreng and fresh leaves of *M. oppositifolia* L. were carefully washed, cut and dried at 50°C. The fruits and leaves were powdered (100 g) and mixed with 500 mL of 80% ethanol in round bottomed Erlenmeyer flask, soaked for 48 h and filtered. Using a rotary evaporator (Heidolph^{R} Rotary Evaporator, Schwabach, Germany) filtrated extracts were concentrated and the ethanol was removed. The percent yield of extract was calculated by dividing the weight of the extract by the weight of plant material multiply by 100.

Phytochemical screening for flavonoids

One hundred gram of air-dried fruits of *A. bunius* (L.) Spreng and leaves of *M. oppositifolia* L. were ground to fine powder, soaked in 80% ethanol for 48 h, filtered and concentrated *in vacuo*. The presence of flavonoids was determined by conducting the Bate-Smith and Metcalf method and Wilstatter “cyanidin” test as previously described (Guevarra, 2005) and utilized (Doctor and Manuel, 2014). Briefly, Bate-Smith and Metcalf method was carried out with the treatment of the ethanolic extract with hydrochloric acid (HCl) while the Wilstatter “cyanidin” test was carried out by using 1% aluminium chloride solution in methanol concentrated HCl, magnesium turnings and potassium hydroxide solution (Guevarra,

2005).

Acute toxicity test

Eighty mice of both sexes were grouped into four with five mice per group. The fresh extracts were prepared by obtaining 250 g fresh fruits of *A. bunius* (L.) Spreng and 250 g fresh leaves of *M. oppositifolia* L. The fresh leaves were osterized and filtered with a clean cheese cloth. The fresh extracts were placed in clean bottles and were administered orally using a gavage at the dose of 6.25, 12.5, 18.75 and 25 ml/kg. The ethanolic extracts were given at 100, 250, 500 and 1000 mg/kg. Mice were given feed and water *ad libitum*. Signs of toxicity and percentage mortality were observed over 48 h.

Treatments

The db/db diabetic male mice were randomly divided into five groups consisting of five mice each. The mice in the first group were given distilled water and served as the diabetic control. The second and third groups were given fresh and ethanolic fruit extracts of *A. bunius* (L.) Spreng at a concentration of 12.5 ml/kg and 250 mg/kg, respectively. The fourth and fifth groups were given fresh and ethanolic leaf extracts of *M. oppositifolia* L. at a concentration of 12.5 ml/kg and 250 mg/kg, respectively. Fruit and ethanolic extracts were administered via gavage daily (at 1700 h).

Data collection

The blood glucose levels were measured on day 1 (D1), day 8 (D8), day 15 (D15), day 22 (D22) and day 29 (D29). Blood samples were collected from the tail vein and measured with a glucometer (OneTouch^{R} Ultra^{R}, LifeScan, Inc., Milpitas, CA, USA). The reduction in blood glucose levels was calculated by subtracting the blood glucose level of mouse on D22 or D29 from D1, and dividing it to the glucose level on D1 multiply by 100.

Statistical analysis

All data were expressed as mean ± standard error of mean (SEM). The effect of *A. bunius* (L.) Spreng and *M. oppositifolia* L. fresh and ethanolic extracts on blood glucose levels was determined using one way-analysis of variance (Graph Pad In Stat, Graph Pad Software Inc., San Diego, CA, USA) followed by post-hoc Tukey-Kramer multiple comparisons test.

RESULTS

Percentage yield and phytochemical screening of *A. bunius* (L.) Spreng and *M. oppossitifolia* L.

The yield of the ethanolic fruit extract of *A. bunius* (L.) Spreng was 13.52% (w/w) dry matter and was reddish purple in color while the ethanolic leaf extract of *M. oppositifolia* L. was 11.73% (w/w) dry matter and was dark green in color. Using the Bate-Smith and Metcalf method, and Wilstatter “cyanidin” test (Guevarra, 2005; Doctor and Manuel, 2014) revealed that flavonoids were detected in ethanolic extracts of *A. bunius* (L.) Spreng and *M. oppositifolia* L.

Acute toxicity test

The acute toxicity test of fruit and leaf extracts of *A. bunius* (L.) Spreng and *M. oppositifolia* L. in mice did not produce mortality or signs of toxicity even with the highest dose of extract (1000 mg/kg) administered orally throughout the observation period of 48 h. The absence of mortality or signs of toxicity indicates that the fresh and ethanolic fruit or leaf extract as well as the dose used in the present study were safe in animals.

Hypoglycemic effect of *A. bunius* L and *M. oppossitifolia* L

Data on the blood glucose levels of db/db mice following treatment with *A. bunius* (L.) Spreng and *M. oppositifolia* L. fresh and ethanolic extracts are presented in Figure 1.

The results showed that from D1 to D15, no significant differences were observed between groups on the blood glucose levels in db/db mice treated with *A. bunius* (L.) Spreng and *M. oppositifolia* L. fresh and ethanolic extracts. However, on D22 a significant reduction of blood glucose levels in db/db mice treated with *M. oppositifolia* L. fresh extract was evident compared with the untreated db/db diabetic mice ($P < 0.05$). On D29, a significant reduction in blood sugar levels was observed in db/db mice treated with *A. bunius* (L.) Spreng fresh extract ($P<0.01$) and *M. oppositifolia* L. fresh ($P<0.01$) and ethanolic extracts ($P < 0.05$) compared with untreated db/db diabetic mice. These decreased the blood glucose levels by 79% with *A. bunius* (L.) Spreng fresh extract, 79 and 55%, with *M. oppositifolia* L. fresh and ethanolic extracts, respectively (Table 1).

DISCUSSION

The hypoglycemic activity of *A. bunius* (L.) Spreng and *M. oppositifolia* L. was evaluated in db/db mouse model of diabetes. The present results confirm previous investigations demonstrating that *A. bunius* (L.) Spreng exhibits antidiabetic activity in streptozotocin- (Chowtivannakul et al., 2016) and in alloxan- (El-Tantawy et al., 2015) induced diabetic rats and further extend the findings on the antidiabetic activity of *A. bunius* (L.) Spreng in a mouse model of diabetes, db/db mice. Importantly, the present results show that *M. oppositifolia* L. exhibits antidiabetic activity in db/db mice. To our knowledge, this is the first *in vivo* evidence showing the antidiabetic activity of *M. oppositifolia* L. in db/db mice.

Consistent with previous reports, hyperglycemia is evident in db/db mice (Huynh et al., 2012). In the present study, untreated db/db mice all exhibited marked hyperglycemia throughout the duration of the study. Specifically on D29, untreated db/db mice had significantly higher blood glucose levels compared with db/db mice treated with *A. bunius* (L.) Spreng fresh

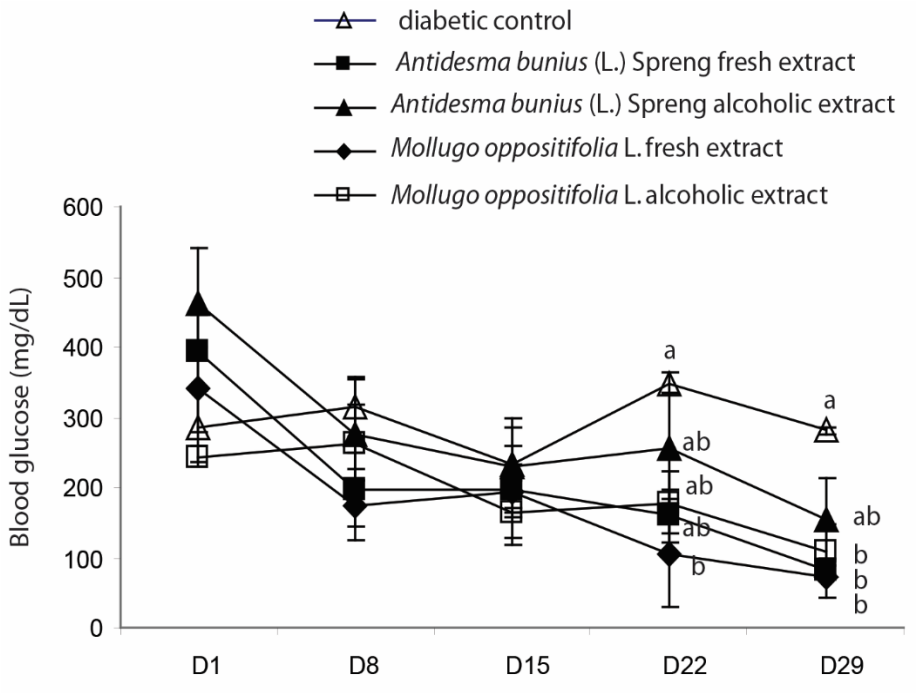

Figure 1. Blood glucose of untreated db/db diabetic control and db/db diabetic mice treated with fresh and ethanolic extracts of *Antidesma bunius* (L.) Spreng and *Mollugo oppossitifolia* L. (n=5). Values are means ± SEM. Values with different letters are significantly different from each other in each time point studied (Tukey–Kramer multiple comparisons test after ANOVA). D1, Day 1; D8, Day 8; D15, Day 15; D22, Day 22; D29, Day 29.

Table 1. Percentage reduction of blood glucose levels following *Antidesma bunius* L and *Mollugo oppossitifolia* L treatment in db/db mice.

Treatment	Blood glucose (mg/dL)			Percentage reduction	
	Day 1	Day 22	Day 29	Day 22	Day 29
Diabetic control	286.2	348.6	280.8	-	-
Antidesma bunius fresh extract	393	159.2	82.4	59.5	79.0
Antidesma bunius alcoholic extract	462.2	256.2	154.8	44.6	66.5
Mollugo oppossitifolia fresh extract	339.4	106.2	71	66.4	79.1
Mollugo oppossitifolia alcoholic extract	243.4	178.4	109.6	26.7	55.0

extract and *M. oppositifolia* L. fresh and ethanolic extracts. The potential mechanism by which *A. bunius* (L.) Spreng and *M. oppositifolia* L. reduced the blood glucose level is not clearly defined. Given that the present study was limited to investigating whether *A. bunius* (L.) Spreng and *M. oppositifolia* L. demonstrate an anti-diabetic potential in a mouse model of diabetes, the specific mechanisms by which *A. bunius* (L.) Spreng and *M. oppositifolia* L. decrease the elevated blood glucose levels could not be conclusively described. However, based on previous studies, we proposed that *A. bunius* (L.) Spreng and *M. oppositifolia* L. could lower blood glucose levels by increasing insulin input or inhibiting the intestinal absorption of glucose (Marviya et al., 2010). These potential mechanisms could lead to the restoration of the pancreatic tissue function (Marviya et al., 2010), and consequently lowers the elevated blood glucose level. Moreover, it is likely that *A. bunius* (L.) Spreng and *M. oppositifolia* L. may have attenuated death of β cells in the db/db mouse since the use of medicinal plant in this strain was associated with significant increases in insulin content accompanied with preservation of β cell architecture (Huynh et al., 2012). Therefore, protection of functional β cell would also mean preservation of insulin production, thereby decreasing the elevated blood glucose level.

Medicinal plants containing active biological principles including flavonoids, and tannins have been reported to demonstrate hypoglycemic properties (Suba et al., 2004). In particular, the therapeutic potential of flavonoids displays significant insulin secretogogue, insulinomimetic and cytoprotective effects (Bharucha et al., 2011). Considering that *A. bunius* (L.) Spreng contains flavonoids (Butkhup and Samappito, 2008) and *M. oppositifolia* L. contains C-glycosyl-flavonoids (Chopin et al., 1984), it is tempting to speculate that the active biological principles present in the plant may be responsible for the hypoglycemic effects observed in the present study.

Conclusion

Overall, the present study provides the first physiological evidence that *A. bunius* (L.) Spreng and *M. oppositifolia* L. possess significant antidiabetic activity in db/db diabetic mouse model. The results may offer a valuable therapeutic potential in the treatment of diabetes mellitus; however, further investigations are required to address the exact mechanism by which *A. bunius* (L.) Spreng and *M. oppositifolia* L. lower elevated blood glucose levels in diabetic mice.

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REFERENCES

Bharucha B, Dwivedi M, Laddha NC, Begum R, Hardikar AA, Ramachandran AV (2011). Antioxidant rich flavonoids from Oreocnide integrifolia enhance glucose uptake and insulin secretion and protects pancreatic beta-cells from streptozotocin insult. BMC Complementary and Alternative Medicine 11:126.

Butkhup L, Samappito S (2008). An analysis on flavonoids contents in Mao Luang fruits of fifteen cultivars (*Antidesma bunius*), grown in northeast Thailand. Pakistan Journal of Biological Science 11:996-1002.

Chopin J, Dellamonia G, Markham KR, Ramachandran Nair A, Gunasegaran R (1984). 2"-p-coumaroylvitexin 7-glucoside from Mollugo oppositifolia. Phytochemistry 23:2106-2108.

Chowtivannakul P, Srichaikul B, Talubmook C (2016). Hypoglycemic and Hypolipidemic Effects of Seed Extract from *Antidesma bunius* (L.) Spreng in Streptozotocin-induced Diabetic Rats. Pakistan Journal of Biological Science 19:211-218.

Doctor TR, Manuel JF (2014). Phytochemical screening of selected indigenous medicinal plants of Tublay, Benguet Province, Cordillera Administrative Region, Philippines. International Journal of Scientific and Research Publications 4(4).

El-Tantawy WH, Soliman ND, El-naggar D, Shafei A (2015). Investigation of antidiabetic action of *Antidesma bunius* extract in type 1 diabetes. Archives of Physiology and Biochemistry 121(3):116-122.

Ferreira AP, Soares GL, Salgado CA, Goncalves LS, Teixeira FM, Teixeira HC, Kaplan MA (2003). Immunomodulatory activity of *Mollugo verticillata* L. Phytomedicine: International Journal of Phytotherapy Phytopharmacology 10:154-158.

Guevarra BQ (2005). A guidebook to phytochemical screening: phytochemical and biological. Manila, Philippines: UST Publishing House.

Hajdu Z, Hohmann J (2012). An ethnopharmacological survey of the traditional medicine utilized in the community of Porvenir, Bajo Paragua Indian Reservation, Bolivia. Journal of Ethnopharmacol 139:838-857.

Huynh K, Kiriazis H, Du XJ, Love JE, Jandeleit-Dahm KA, Forbes JM, McMullen JR, Ritchie RH (2012). Coenzyme Q10 attenuates diastolic dysfunction, cardiomyocyte hypertrophy and cardiac fibrosis in the db/db mouse model of type 2 diabetes. Diabetologia 55:1544-1553.

IDF Diabetes Atlas Eight Edition (Published 2017). Accessed June 28, 2019). http://www.idf.org/diabetesatlas

Kim SS, Kim JY, Lee NH, Hyun CG (2008). Antibacterial and anti-inflammatory effects of Jeju medicinal plants against acne-inducing bacteria. Journal of General and Applied Microbiology 54:101-106.

Lin CC, Ng LT, Yang JJ (2004). Antioxidant activity of extracts of peh-hue-juwa-chi-cao in a cell free system. The American Journal of Chinese Medicine. 32:339-349.

Morton J (1987). Fruits of warm climates. In: Bignay, vol. 2012: http://www.hort.purdue.edu/newcrop/morton/bignay_ars.html, Miami, FL.

Marviya N, Jain S. Malviya S. (2010). Antidiabetic potentials of medicinal plants. Acta Poloniae Pharmaceutica ñ Drug Research 67(2):113-118.

Padma P, Khosa RL (1995). Identity of Mollugo stricta roots: A potential antifertility drug for future. Ancient science of life 15(2):97.

Quisumbing E (1978). Medicinal Plants of the Philippines. Quezon City, Philippines: Katha Publishing This book is available in the Philippines as a hard copy.

Rajasekaran M, Nair AG, Hellstrom WJ, Sikka SC (1993). Spermicidal activity of an antifungal saponin obtained from the tropical herb Mollugo pentaphylla. Contraception 47:401-412.

Reyes BAS, Dufourt EC, Ross J, Tanquilut NC, Leung AB (2017). Antidiabetic properties of the bioactive compounds in natural products. In: Bioactive Natural Products(Atta-Ur-Rahman, ed) Amsterdam: Elsevier Science Publishers.

Rosak C (2002). The pathophysiologic basis of efficacy and clinical experience with the new oral antidiabetic agents. Journal of Diabetes Complications 16:123-132.

Sarah W, Gojka R, Anders G, Richard S, Hilary K (2004). Global prevalence of diabetes estimates for the year 2000 and projections for 2030. Diabetes Care 27:1047-1053.

Searls Y, Smirnova IV, Vanhoose L, Fegley B, Loganathan R, Stehno-Bittel L (2012). Time-dependent alterations in rat macrovessels with type 1 diabetes. Experimental Diabetes Research 2012:278620.

Suba V, Murugesan T, Rao RB, Ghosh L, Pal M, Mandal SC, Saha BP (2004). Antidiabetic potential of Barleria lupulina extract in rats. Fitoterapia 75:1-4.

Sun JE, Ao ZH, Lu ZM, Xu HY, Zhang XM, Dou WF, Xu ZH (2008). Antihyperglycemic and antilipidperoxidative effects of dry matter of culture broth of Inonotus obliquus in submerged culture on normal and alloxan-diabetes mice. Journal of Ethnopharmacology 118:7-13.

Udomkasemsab A, Ngamlerst C, Adisakwattana P, Aroonnual A, Tungtrongchitr R, Prangthip P (2018). Maoberry (*Antidesma bunius*) ameliorates oxidative stress and inflammation in cardiac tissues of rats fed a high-fat diet. BMC Complementary and Alternative Medicine 18:344.

Villasenor IM, Lamadrid MR (2006). Comparative anti-hyperglycemic potentials of medicinal plants. Journal of Ethnopharmacology 104:129-131.

Yki-Jarvinen H (1994). Pathogenesis of non-insulin-dependent diabetes mellitus. Lancet 343:91-95.

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Endophytic fungi of different species have already proved their efficacy against a range of pathogenic bacteria. However, the separation of the compounds contained in these extracts that actually have an effective activity is not a constant, but it is essential for the scientific knowledge to formulate a new medication. Thus, this study aimed to separate compounds from two endophytic fungi extracts of *Uncaria tomentosa* with positive results against Gram-positive and Gram-negative bacteria. The separation was carried out by thin-layer chromatography and bioautography. For this, it was necessary to produce fungal extracts from the endophytes Colletotrichum (23916) and Fusarium (23952), which were separated from the liquid state by filtration. The remaining filtrate was divided into ethyl acetate and then concentrated in a rotary evaporator at 40°C. Antimicrobial activity was evaluated and the Minimum Inhibitory Concentration (MIC) was determined with the following test microorganisms: *Escherichia coli, Enterecoccus faecalis, Klebsiella pneumoniae* and *Staphylococcus aureus*. Subsequently, it was carried out the thin layer chromatography (TLC) of the extracts using hexane, ethyl acetate/hexane, ethyl acetate and acetate/methanol. The reading was performed in 312 nm UV light, and right after the chromatography, the bioautography was carried out. The following Rf values were obtained: 0.30 for K. pneumoniae and 0.35 for *E. coli*, in ethyl acetate, for the extract 23952, and 0.81 for *K. pneumoniae* and 0.41 for *S. aureus*, in styrene acetate, for the extract 23916, confirming different compounds by bioautography. Therefore, it was concluded that several compounds present in the extracts tested have antibacterial activity.

Key words: Chromatography, bioautography, endophytic.

INTRODUCTION

Thin Layer Chromatography (TLC) is a technique used to separate compounds of a sample of a given substance using two phases, a stationary phase and a mobile one, on silica gel plates (Niessem, 2017).

The scope of its applicability is the identification of molecular compounds by comparing them with previously existing standards, separating the components of a mixture through the purification technique (Bonnin et al.,

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2018). Bioautography is a technique that combines thin-layer chromatography with agar diffusion (Choma and Jesionek, 2015). Both techniques are tools for the separation of molecules at laboratory level, capable of biologically identifying which specific compounds of an extract act actively in that compound, such as the endophytic fungi extracts from several plant species that act as potent antimicrobials, since some known drugs and microorganism derivatives (Fanali et al., 2016) are already on the market as antibiotics (Luo et al., 2016), being used in the treatment of various diseases such as Alzheimer's, from bioactive (Wang et al., 2016), anticancer (Petersen et al., 2014; Zaiyou et al., 2015) and anti-allergic compounds, among others (Sakurai et al., 2003).

Among these microorganisms that produce active metabolites, the endophytes that are fungi or bacteria that reside within plant tissues without causing apparent damage (Li et al., 2018) stand out. These are sources of secondary metabolites with many biological activities (Yan et al., 2018). The use of these compounds is already a promising reality in the field of biotechnology, especially in pest control in agriculture, due to their low cost and benefits to human health, especially when compared to the use of agrochemicals (Silva et al., 2008), and are also applied in the medical and pharmacological fields in the inhibition of pathogenic bacteria that are resistant to the main antibiotics currently available in the market (Arora and Kaur, 2018). An example of this is the significant number of compounds produced from fungal endophytes with proven inhibitory activity against several Gram-positive and Gram-negative bacteria (Banhos et al., 2014).

The Brazilian Amazon is a wide source of new compounds from its rich biodiversity for the development of new drugs, mainly from its endophytic fungi (Banhos et al., 2014). Also, the analysis of their biological activity is an appropriate option in the fight against resistant microorganisms; however, there are few studies on this subject in the literature, which becomes a limiting factor in the contribution to the formulation of new drugs (Cheng et al., 2018).

Uncaria tomentosa (Willd.) DC belongs to the Rubiaceae family, it is a plant species of the Amazon region, popularly known as cat's claw (Alvarenga et al., 2018). This species has various medicinal properties such as anti-inflammatory (Yatoo et al., 2018), anticancer (Zhang et al., 2015), contraceptive (Nogueira et al., 2011), antiasthmatic (Azevedo et al., 2018), antiarthritic (Rajamanikyam et al., 2017), and immunostimulant (Baraya et al., 2017) activities, mainly antibacterial activity of endophytic fungi extracts, especially against *Enterecoccus faecalis* (Rodrigues et al., 2018), which is a Gram-positive bacterium, ubiquitous, highly resistant and commonly found in the root canal system (Qian-Wang et al., 2012). In the contemporary world, the indiscriminate use of antimicrobials is frequent, so they no longer have the same efficacy against a specific group of bacteria, known as superbugs or resistant bacteria (Yelin and Kishony, 2018). Based on the instability of the immune system, several opportunistic bacteria can cause infections and establish acute pathological conditions that lead the host organism to unpleasant consequences (Sedghizadeh et al., 2017), such as *Escherichia coli*, leading to food poisoning and diarrhea (Gomez-Duarte, 2014). *Klebsiella pneumoniae* is another pathogenic bacterium capable of causing pneumonia by different types of transmission, mostly in the hospital environment (Xiong et al., 2016). *Staphylococcus aureus* can cause various infections, such as acne, boils, pneumonia, meningitis, endocarditis, sepsis and others (Koch et al., 2014). Strains resistant to various drugs have become increasingly prevalent (Otto, 2012).

In this context, the antimicrobial activity of endophytic fungi of *U. tomentosa* makes this species a promising source for the development of a new drug. However, it is not entirely known, what compounds or molecules are contained in the endophytic fungi extracts of this plant that actually inhibit bacterial growth. For this reason, the separation of these substances is as important as the extract activity. Thus, this study aimed to separate the compounds of two endophytic fungal extracts of *U. tomentosa*: *Colletotrichum* and *Fusarium* which had positive results against Gram-positive and Gram-negative bacteria from the collection of the microbiology laboratory of the Federal University of Acre (UFAC).

MATERIALS AND METHODS

Obtainment of extracts and purification of endophytic fungi of *U. tomentosa*

The fungi *Fusarium* with laboratory record (23952) and *Colletotrichum* (23916), isolated from *U. tomentosa* of the collection of the microbiology laboratory of the Federal University of Acre (UFAC), with positive results of antibacterial activity were cultivated on a large scale, in a broth using a fermentor to obtain fungal extracts to be used in *in vitro* biological assays. For this purpose, they were cultivated in Petri dishes using potato dextrose agar (PDA) medium for 14 days at 28°C. The content from 5 dishes (80 × 15 mm) with the grown fungus was inoculated into a 1000 ml Erlenmeyer flask containing 500 ml of PD broth and then conducted for 14 days at 28°C without shaking. For each fungus, 3000 ml of PD broth was used and distributed into six Erlenmeyer flasks of 1000 ml each. After incubation, the fungal mycelium was separated from the broth by filtration using filter paper. The filtrate was subjected to the liquid-liquid partition with 200 ml of ethyl acetate five times, and then the extraction product was concentrated on a rotary evaporator under reduced pressure in a water bath at 40°C (Cechinel and Yunes, 1998) until the dry yield was calculated dividing the weight value of two dry extracts by the weight of empty flasks, and solubilizing them with Dimethylsulfoxide (DMSO) for the antibacterial activity bioassay and determination of the Minimum Inhibitory Concentration (MIC).

Bioassays of antibacterial activity

The antibacterial activity of the endophytic fungi *Fusarium*

Table 1. Mean and standard deviation of the halos of the antibacterial activity of endophytic fungi extracts isolated from *U. tomentosa*: Colletotrichum 2.3916 and Fusarium 2.3952 against Gram-positive and Gram-negative bacteria.

Extract (EtOAc)	Antibacterial activity (mm)			
	E. coli	***S. aureus***	***K. pneumonia***	***E. faecalis***
Colletotrichum 2.3916	10.3 ±0.57	13.0±2.6	15 mm±1.0	13.6±0.5
Fusarium 23952	12.3±0.57	11.6±1.5	14.3±2.0	13.3±1.5

Table 2. MIC of endophytic fungi extracts *of U. tomentosa against Escherichia coli, S. aureus, K. pneumoniae,* and *E. faecalis.*

Extract	Dry matter yield (mg)	Susceptibility of bacteria mg/mL			
		E. coli	***S. aureus***	***K. pneumoniae***	***E faecalis***
(EtOAc)		**MIC**	**MIC**	**MIC**	**MIC**
Colletotrichum 2.3916	49.83	31.25	7.81	7.81	7.81
Fusarium 23952	32.12	31.25	7.81	7.81	15.62

(23952) and *Colletotrichum* (23916) was evaluated using the well method for the allocation of extract, according to the National Committee for Clinical Laboratory Standards, using the following test-microorganisms: *E. coli* (ATCC 10536)*, S. aureus* (ATCC 12598)*, K. pneumoniae* (ATCC 700603) and *E. faecalis* (ATCC 4083). For this, Petri dishes containing Müeller-Hinton agar medium were prepared and incubated at 37°C for 24 h to verify the sterility of the medium, where the bacterial purification process was carried out, using the streak plate technique. Afterward, they were incubated at 37°C for 24 h. Five isolated bacterial colonies were spread in Luria Bertani broth (LB) and then incubated for up to 3 h. The turbidity degree was then checked and adjusted to the McFarland 0.5 scale (1.5×10^8 cells/ml) with a sterile saline solution. After adjustment, the bacteria were spread onto Petri dishes containing Müeller-Hinton agar medium using a swab in three distinct directions and around the dish uniformly. Prior to the inoculation of the bacteria, wells of approximately 5 mm in diameter were made in pipette tips to place the extract. After spreading the test-bacteria on the wells, 20 µl of the fungal extracts were added. Subsequently, the dishes were incubated at 37°C for 24 h and then the formation of the inhibition halo was observed. Samples considered to have positive antibacterial activity were those that did not allow the microbial development around the well, and these halos were measured in millimeters. A millimetre (mm) graduated ruler was used to measuring the size of the halos obtained from the well diffusion bioactivity test.

Determination of minimum inhibitory concentration

After obtaining the dried fungal extracts, they were solubilized in dimethyl sulfoxide (DMSO) at an initial concentration of 1, 250, 125, 62.5, 31.25, 15.62, 7.81 and 3.90 mg mL^{-1}. After the solubilization of the extracts in the respective concentrations, the *in vitro* antibacterial bioactivity test against *E.coli, S. aureus, K. pneumoniae,* and *E. faecalis* was performed to determine MIC, by the well method in Müeller-Hinton medium; then the extracts were applied at their respective concentrations (Montero, 2017).

Chromatography and bioautography of the tested fungal extracts

After obtaining the results of the bioactivity test against *E. faecalis*, the extracts were subjected to the chromatography process to separate the compounds with biological activity. For this, 10 µl of each extract were placed on the plates of TLC (Silica gel 60), eluted in the mobile phase using different low, medium and high polarity systems: hexane, ethyl acetate-hexane, ethyl acetate (EtOAc) (1:1), ethyl acetate/methanol (EtOAc/MeOH) (1:1), and analyzed in ultraviolet (UV), 312 nm. Soon afterward, to perform the bioautography, each TLC plate was placed on dishes containing Müeller-Hinton medium, properly identified according to each solvent used, and stored for 24 hours in a refrigerator. After this period, the test-bacteria were spread and incubated in a bacteriological oven at 37°C for 24 h. Then, the inhibition zone of the bioactive compounds was analyzed, as described above, and the retention factors (Rf) (Di Ciaccio et al., 2018) were calculated by measuring the distance traveled by the compound (DC) divided by the distance traveled by the solvent (DS) (Kagan and Flythe, 2014).

Data analysis

The data were analyzed from the mean and standard deviation of the halos obtained from the antibacterial activity test, which were performed in triplicate, in addition to the calculation to measure the retention factors (Rf) of the compounds with the equation: (Rf = DC / DS).

RESULTS

The antimicrobial activity was positive for the two fungal extracts tested, *Fusarium* (23952) and *Colletotrichum* (23916), for all bacteria tested: *E. coli, S. aureus, K. pneumoniae* and *E. faecalis* with formation of inhibition halo measured in mm (Table 1) and MIC of 7.81 for the majority of the bacteria tested for the two extracts, except for *E. coli* with 31.25, for both extracts, and for *E. faecalis* with 15.62, for extract 23952 (Table 2).

From the reading of the UV chromatographic plates, several components were found in the extracts used in this study and, according to the bioautography, these substances have high antibacterial potential (Figure 1). The bioautography results confirm antimicrobial activity

for *Fusarium* fungal extract with values of Rf (0.30) for *K. pneumoniae* in ethyl acetate eluent and 0.85 for acetate/methanol; Rf (0.74) for *S. aureus* in hexane/ethyl acetate eluent and 0.35 for ethyl acetate; Rf (0.67) for *E. coli* in ethyl acetate/methanol and Rf (0.64) for *E. faecalis* in ethyl acetate/methanol (Figure 1). For the *Colletotrichum* extract, the following Rf values were obtained: 0.81 against *K. pneumoniae*, 0.41, 0.36 and 0.45, all in ethyl acetate, for *S. aureus*, *E. coli* and *E. faecalis*, respectively, and 0.63 *E. coli*, in ethyl acetate/methanol.

DISCUSSION

The antibacterial activity of endophytic fungi of several plant species is already described in the literature from many works in the field of biotechnology (Sudha et al., 2016) as it can be observed in studies on extracts of endophytic fungi of *Ipomea biloba*, which showed positive result against pathogenic bacteria (Prakash et al., 2016) as well as on extracts of endophytic fungi from *Azadirachta indica* A. Juss, which have proved to be potent antibacterial agents (Marcinkevicius et al., 2019). In this context, the antibacterial activity of extracts of endophytic fungi (*Colletotrichum* 23916 and *Fusarium* 23952) based on ethyl acetate from *U. tomentosa* presented in this study was confirmed, proving to be effective against bacteria, both for Gram-positive and Gram-negative bacteria.

The endophytic fungi *Colletotrichum* and *Fusarium* are considered sources of defense metabolites with several biological activities, mainly with antibacterial activity (Juang et al., 2019), corroborating the results found in this study. The MIC is the lowest concentration of the extract capable of stopping the growth of a microorganism (Tripathi, 2013). In this study, the MIC was 7.81 for both fungal extracts for most of the bacteria tested, except for *E. coli* (31.25) and *E. faecalis* (15.62) for the *Fusarium* 23952 extract, similarly to the results of other studies on MIC determination in antimicrobial activity (Nisa, 2015; Ochoa-Velasco et al., 2018; Naime-Shamel et al., 2019).

Thin-layer chromatography reveals that from a stationary and a mobile phase it is possible to separate compounds that travel the entire surface of the plate (TLC), according to the degree of polarity of the compound and solvent used (Agostini-Costa et al., 2012). In addition to TLC, there are other types of chromatography, such as gas chromatography and liquid diffusion (Marathe et al., 2019). The advantage of TLC is the adsorption (Kalindere et al., 2018), for this reason, the TLC was chosen in this research.

In this study, it was demonstrated that the fungal extracts used for both *Colletotrichum* 23916 and *Fusarium* 23952 have different UV-visible compounds, which are mostly of medium polarity with better separation in ethyl acetate. Similar results are presented in other studies using chromatography (Li et al., 2019; Marcinkevicius et al., 2019), demonstrating the appearance of different compounds in ethyl acetate. Ethyl acetate is a solvent that has medium polarity and has an affinity with adsorptive silica (polar) molecules, and this facilitates the separation of the compounds from an extract (Sui et al., 2018). Recalling that nonpolar compounds have greater difficulty in silica plates with acidic and basic solvents (Churms, 2002). On the other hand, some authors question the fidelity of the chromatography information due to the spreading of the fractions and colonization of the TLC plates (Mccalley, 2010). The solvents used in the mobile phase may have an effect on bacterial growth and may interfere with the results (Arshad et al., 2018). However, correct drying of TLC plates to eliminate solvents is necessary for the effectiveness of bioautography (Choma and Jesionek, 2015), even though the technique of plate bioautography by evaluating the mark left by diffusion is considered a reliable method of confirmation (Choma and Grzelak, 2011).

The bioautography technique associates thin layer chromatography with the confirmation of the antibacterial activity of the compounds separated by TLC from antimicrobial analysis on plates containing Müller Hilton agar (Matanna et al., 2010). The bioautography can be performed using different techniques, diffusion and dilution (Choma and Grzelak, 2011), but the use of the mark in solid state was chosen because it is a practical technique, validated for microbiological control in the laboratory, that is, to evaluate the antimicrobial substance of a mixture, simultaneously with its quantification regarding the activity of a standard (Colorado et al., 2007).

The results of the bioautography obtained in this study confirmed that the compounds revealed in TLC have antibacterial activity against the bacteria tested. These results are in agreement with other studies on chromatography and bioautography reported in the literature (Chen and Schwack, 2014), in which bioactive compounds were found in fungal extracts of *Terminalia arjuna* (Gill and Vasundhara, 2019). Similarly, more compounds with antibacterial activity were also isolated in endophytic fungi extracts of *Helianthus annuus* (Farhat et al., 2019).

Conclusion

According to the *in vitro* assays and the chromatographic and bioautographic analysis performed to separate the chemical compounds from the extract, with subsequent verification of the retention factors (Rf), there are different compounds in the fungal extracts (*Colletotrichum* 23916 and *Fusarium* 323952) with an inhibition reaction of bacterial growth in all samples tested, especially with

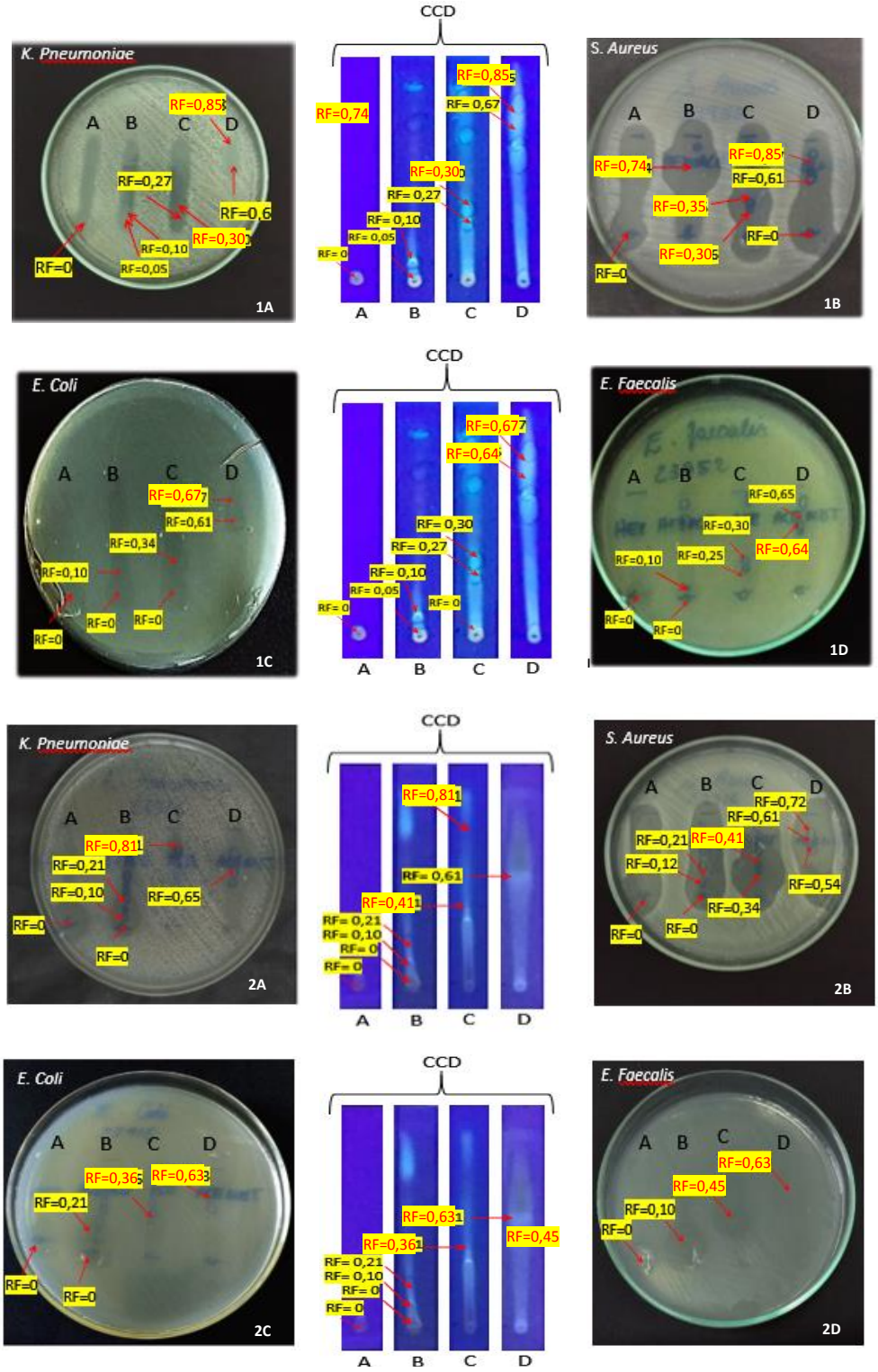

Figure 1. Bioautography of fungi metabolites (*Fusarium* 23952 and *Colletotrichum* 23916) with antibacterial activity. Figure 1A-1D (*Fusarium* 23952), respectively, in the following order of bacteria test: *K. pneumoniae, S. aureus, E. coli* and *E. faecalis*. Figure 2A-2D (*Colletotrichum* 23916), respectively, in the following order of bacteria test*: E. coli, S. aureus, K. pneumoniae* and *E. faecalis,* TLC (thin layer chromatography) of A-D in Figures 1 and 2, respectively, represent (A) eluent system in hexane, (B) eluent system ethyl acetate/hexane (1:1), (C) eluent system in ethyl acetate and (D) eluent system in ethyl acetate/methanol (1:1). Rf = retention factor.

ethyl acetate solvent.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Alvarenga-Venutolo S, Rosales-López C, Sánchez-Chinchilla L, Muñoz-Arrieta R, Aguilar-Cascante F (2018). Seasonality effect on the composition of oxindole alkaloids from distinct organs of Uncaria tomentosa from the Caribbean region of Costa Rica. Phytochemistry 151:26-31.

Arora DS, Kaur N (2019). Antimicrobial Potential of Fungal Endophytes from *Moringa oleifera*. Applied Biochemistry and Biotechnology 187(2):628-648.

Arshad M, Qayyum A, Shar GA, Soomro GA, Nazir A, Munir B, Iqbal M (2018). Zn-doped SiO2 nanoparticles preparation and characterization under the effect of various solvents: Antibacterial, antifungal and photocatlytic performance evaluation. Journal of Photochemistry and Photobiology B: Biology 185:176-183.

Azevedo BC, Morel LJF, Carmona F, Cunha TM, Contini SHT, Delprete PG, Borges MC (2018). Aqueous extracts from Uncaria tomentosa (Willd. ex Schult.) DC. reduce bronchial hyperresponsiveness and inflammation in a murine model of asthma. Journal of Ethnopharmacology 218:76-89.

Banhos EFD, Souza AQLD, Andrade JCD, Souza ADLD, Koolen HHF, Albuquerque PM (2014). Endophytic fungi from Myrcia guianensis at the Brazilian Amazon: distribution and bioactivity. Brazilian Journal of Microbiology 45(1):153-162.

Baraya YS, Wong KK, Yaacob NS (2017). The Immunomodulatory Potential of Compounds Based on Bioactive Plants Selected in Breast Cancer: Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 17(6):770-783.

Bonnin RR, Reverter FC, Poblador SE, Tomás FJG, de la Presa BG, Martínez RML (2018). Development of measurement procedures based on high performance liquid chromatography. Journal of the clinical Laboratory 11(3):137-146.

Cechinel FV, Yunes RA (1998). Estratégias para a obtenção de inventariados farmacológicos a partir de plantas medicinais: conceitos sobre modificações estruturais para a otimização da atividade. Quim. Nova 21(1):99-105.

Chen Y, Schwack W (2014). High-performance thin-layer chromatography screening of multi class antibiotics in animal food by bioluminescent bioautography and electrospray ionization mass spectrometry. Journal of Chromatography A 1356:249-257.

Cheng X, Wei Z, Pu S, Xiang M, Yan A, Zhang Y, Wang X (2018). Diversity of endophytic fungi of Paeonia lactiflora Pallas and screening for fungal paeoniflorin producers. FEMS microbiology letters 365(24):fny263. https://doi.org/10.1093/femsle/fny263.

Choma I, Jesionek W (2015). Effects-Directed Biological Detection. Instrumental Thin-Layer Chromatography pp. 279-312. doi:10.1016/b978-0-12-417223-4.00011-x

Choma IM, Grzelak EM (2011). Bioautography detection in thin-layer chromatography. Journal of Chromatography A, 1218(19):2684-2691.

Churms SC (2002). High Performance Hydripjilic Interaction Chromatography of Carboydrates with Polar Sorbents. Journal of Chromatography Library 66:121-163.

Colorado J, Galeano E, Martínez A (2007). Desarrollo de la bioautografía directa como método de referencia para evaluar la actividad antimicrobiana de la gentamicina contra Escherichia coli. Scielo. Vitae 14(1) Medellín Jan./June. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0121-40042007000100010&lang=pt

Fanali C, Dugo L, Mondello L (2016). Advances in Chromatographic Techniques for Food Authenticity Testing. Advances in Food Authenticity Testing, (pp. 253-284). Woodhead Publishing pp. 253-284. https://doi.org/10.1016/B978-0-08-100220-9.00010-2.

Farhat H, Urooj F, Tariq A, Sultana V, Ansari M, Ahmad U, Ehteshamul-Haque S (2019). Avaliação do potencial antimicrobiano de fungos endofíticos associados a plantas sadias e caracterização de compostos produzidos por Cephalosporium e Fusarium solani endofíticos. Biocatálise e Biotecnologia Agrícola, 101043. doi: 10.1016 / j. bcab.2019.101043

Gill H, Vasundhara M (2019). Isolation of taxol producing endophytic fungus *Alternaria brassicicola* from non-Taxus medicinal plant *Terminalia arjuna*. World Journal of Microbiology and Biotechnology 35(5). doi:10.1007/s11274-019-2651-8

Gómez-Duarte OG (2014). Enfermedad diarreica aguda por *Escherichia coli* enteropatógenas en Colombia. Revista chilena de infectología 31(5):577-586.

Jiang CX, Li J, Zhang J-M, Jim X-J, Yu B, Fang J, Wu Q-X (2019). Isolation, Identification, and Activity Evaluation of Chemical Constituents from Soil Fungus *Fusarium avenaceum* SF-1502 and Endophytic Fungus *Fusarium proliferatum* AF-04. Journal of Agricultural and Food Chemistry 67(7):1839-1846

Kagan IA, Flythe MD (2014). Thin-layer Chromatographic (TLC) Separations and Bioassays of Plant Extracts to Identify Antimicrobial Compounds. Journal of Visualized Experiments (85):e51411

Kalindere Z, Karatuna O, Kozagoz T (2018). Rapid detection of beta-lactamase production including carbapenemase by thin layer chromatography. Journal of Microbiological Methods 156:15-19.

Koch G, Ana Y, Konrad UF, Charlotte W, Stephanie TS, Jennifer M, Knut O, Kevin RF, Daniel L (2014). Evolution of resistance to a last-resort antibiotic in Staphylococcus aureus via bacterial competition. Cell 158(5):1060-1071.

Li JL, Xin J, Xinpei L, Caiwei H, Yuxin D, Saijian L, Huilian H, Bin L, Dong W, Boyi F (2019). Antibacterial anthraquinone dimers from marine derived fungus *Aspergillus* sp. Fitoterapia 133:1-4.

Li SJ, Zhang X, Wang XH, Zhao CQ (2018). Novel natural compounds from endophytic fungi with anticancer activity. European Journal of Medicinal Chemistry 156:316-343. https://doi.org/10.1016/j.ejmech.2018.07.015.

Luo H, Dan X, Rushan X, Xuping Z, Jian W, Xuejiao D, Daowan L, Ligang Z, Yang L (2016). Enhancement of botrallin and TMC-264 production in liquid culture of endophytic fungus *Hyalodendriella* sp. Ponipodef12 after treatments with metal ions. Electronic Journal of Biotechnology 24:12-20.

Maheshwari R, Varahalarao V, Ramars A, Suryanarayana MU (2017). Fungos endofíticos como novos recursos da terapêutica natual. Brazilian Archives of Biology and Technology 60:e17160542.

Marathe PS, Juan A, Xun H, Westerhof RJM, Kersten SRA (2019). Evaluating quantitative determination of levoglucosan and hydroxyacetaldehyde in bio-oils by gas and liquid chromatography. Journal of Analytical and Applied Pyrolysis 139:233-238.

Marcinkevicius K, Salvatore A, Murua G, Arena M, Vera N (2019). Phytochemical investigation and biological activities of Fusarium SP. An entomogenous fungus. Biocatalysis and Agricultural Biotechnology 18:101084.

Mattana CM, Satorres SE, Sosa A, Fusco M, Alcaráz LE (2010). Antibacterial activity of extracts of Acacia aroma against methicillin-resistant and methicillin-sensitive Staphylococcus. Brazilian Journal of Microbiology 41(3):581-587.

Mccalley DV (2010). The challenges of the analysis of basic compounds by high performance liquid chromatography: Some possible approaches for improved separations. Journal of Chromatography A, 1217: 858-880.

Montero M (2017). Efecto Antimicrobiano del Aceite Esencial de Canela (*Cinnamomum zeylanicum*) sobre Cepas de Salmonella. Revista de Investigaciones Veterinarias del Perú 28(4):987-993. http://dx.doi.org/10.15381/rivep.v28i4.13890

Naime-Shamel N, Pourali P, Dolatabadi S (2019). Green Synthesis of

gold nanoparticles using *Fusarium oxysporum* and antibacterial activity of its tetracycline conjugant. Journal de Mycologie Medicale 29(1):7-13.
Niessem WM (2017). Mass Spectrometry: Chromatography–MS, Methods. Encyclopedia of Spectroscopy and Spectrometry pp 758-763. https://doi.org/10.1016/B978-0-12-409547-2.11690-7. 2017
Nisa H (2015). Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: A review. Microbial Pathogenesis Elsevier 82:50-59.
Nogueira NJ, Cavalcante FLLP, Carvalho RAF, Rodrigues TGPDM, Xavier MS, Furtado PGR, Schor E (2011). Contraceptive effect of Uncaria tomentosa (cat's claw) in rats with experimental endometriosis. Acta Cirurgica Brasileira 26:15-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=uncaria+tomentosa+contraceptive.
Ochoa-Velasco CE, Navarro-Cruz AR, Vera-López O, Palou E, Avila-Sosa R (2018). Growth modeling to control (in vitro) Fusarium verticillioides and Rhizopus stolonifer with thymol and carvacrol. Revista Argentina de Microbiología 50(1):70-74.
Otto M (2012). MRSA virulence and spread. Cell Microbiology 14:1513-1521.
Petersen L., Bladt T, Dürr C, Seiffert M, Frisvad J, Gotfredsen C, Larsen T (2014). Isolation, Structural Analyses and Biological Activity Assays against Chronic Lymphocytic Leukemia of Two Novel Cytochalasins—Sclerotionigrin A and B. Molecules 19(7):9786-9797.
Prakash S, Ramasubburayan R, Ramkumar VS, Kannapiran E, Palavesam A, Immanuel G (2016). In vitro—Scientific evaluation on antimicrobial, antioxidant, cytotoxic properties and phytochemical constituents of traditional coastal medicinal plants. Biomedicine and Pharmacotherapy 83:648-657. Elsevier Masson SAS.
Rodrigues RA, de Araujo AV, da Cunha RM, Carvalho CM (2018). Antibacterial activity of endophytic fungi from the medicinal plant Uncaria tomentosa (Willd.) DC. Journal of Medicinal Plants Research 12(15):179-185.
Sakurai M, Nishio M, Yamamoto K, Okuda T, Kawano K, Ohnuki T (2003). TMC-264, um novo heptaketide antialérgico produzido pelo fungo Phoma sp. TC 1674. Organic Letters 5:1083-1085. http://dx.doi.org/10.1021/ol034125v
Sedghizadeh PP, Mahabady S, Allen CM (2017). Opportunistic Oral Infections. Dental Clinics of North America 61(2):389-400.
Silva de FA, Rhavena GL, Ana PAB, Erica de MR, Marilene BSP, Edson LS, Olivia MS (2018). "Diversity of cultivable fungal endophytes in Paullinia cupana (Mart.) Ducke and bioactivity of their secondary metabolites." PloS one 13(4):e0195874.
Sudha V, Govindaraj R, Baskar K, Al-Dhabi NA, Duraipandiyan V (2016). Biological properties of endophytic fungi. Brazilian Archives of Biology and Technology 59:e16150436.
Sui Y, Ma Z, Meng X (2018). Análise proteômica do efeito inibitório do oligochitosana sobre o patógeno fúngico, Botrytis cinerea. Jornal da Ciência da Alimentação e Agricultura.doi: 10.1002 / jsfa.9480
Agostini-Costa TS, Vieira RF, Bizzo HR, Silveira D, Gimenes MA (2012). Secondary Metabolites, Chromatography and Its Applications, Dr. Sasikumar Dhanarasu (Ed.), ISBN: 978-953-51-0357-8, InTech, Available from: http://www.intechopen.com/books/chromatography-and-its- applications/secondary metabolites.
Tripathi KD (2013). Essentials of Medical Pharmacology. 7th ed. New Delhi, India: Jaypee Brothers Medical Publishers pp. 696-697.
Wang QQ, Zhang CF, Chu CH, Zhu XF (2012). Prevalence of Enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis. International Journal of Oral Science 4(1):19.
Wang Z, Ma Z, Wang L, Tang C, Hu Z, Chou G, Li W (2015). Active anti-acetylcholinesterase component of secondary metabolites produced by the endophytic fungi of Huperzia serrata. Electronic Journal of Biotechnology 18(6):399-405.
Xiong H, Keith JW, Samilo DW, Carter RA, Leiner IM, Pamer EG (2016). Innate lymphocyte/Ly6Chi monocyte crosstalk promotes Klebsiella pneumoniae clearance. Cell 165(3):679-689.
Yan L, Zhao H, Zhao X, Xu X, Di Y, Jiang C, Jin M (2018). Production of bioproducts by endophytic fungi: chemical ecology, biotechnological applications, bottlenecks, and solutions. Applied Microbiology and Biotechnology 102(15):6279-6298.
Yatoo M, Gopalakrishnan A, Saxena A, Parray OR, Tufani NA, Chakraborty S, Iqbal H (2018). Anti-inflammatory drugs and herbs with special emphasis on herbal medicines for countering inflammatory diseases and disorders-a review. Recent patents on Inflammation and Allergy Drug Discovery 12(1):39-58.
Yelin I, Kishony R (2018). SnapShot: Antibiotic Resistance. Department of Biology, Technion - Israel Institute of Technology, Haifa 3200003, Israel. Cell 172, Elsevier Inc. DOI http://dx.doi.org/10.1016/j.cell.2018.02.018
Zaiyou J, Hongsheng W, Ning W, Li M, Guifang X (2015). Isolation and identification of an endophytic fungus producing paclitaxel from Taxus wallichiana var mairei. Nutricion Hospitalaria 32(6):2932-2937.
Zhang Q, Zhao JJ, Xu J, Feng F, Qu W (2015). Medicinal uses, phytochemistry and pharmacology of the genus Uncaria. Journal of Ethnopharmacology 173:48-80.

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Full Length Research Paper

Antifungal efficacy of crude extracts of *Azadirachta indica* and *Vernonia amygdalina* against pathogenic A*spergillus niger* (ATCC 16404)

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This research work was conducted to assess the potency of ethanolic and hot water extract of *Azadirachta indica* and *Vernonia amygdalina* against pathogenic *Aspergillus niger* (ATCC 16404). Potato Dextrose Agar was the medium under which the antimicrobial sensitivity test was carried out. Streptomycin solution was also added to the medium to selectively inhibit the growth of bacterial cell. The results showed that, higher concentration of the extracts of the two plants species were efficient in stifling the growth of *A. niger*. This was evident as the inhibition zones increased with increasing concentration of the extracts. The results also showed that ethanolic extract of *A. indica* and *V. amygdalina* is a bit potent than the hot water extracts against the fungus species ATCC 16404. The results revealed that, the areas of inhibitions at the same concentrations were found to be slightly high in ethanolic extracts than that of hot water extracts.

Key words: Antifungal, *Azadirachta indica*, *Vernonia amygdalina*, A*spergillus niger*.

INTRODUCTION

Contamination of stored food by fungi species especially *Aspergillus* species has become a global problem. This problem is more disturbing in the tropics especially in West Africa where the climatic conditions favor the proliferation of these opportunistic fungi. *Aspergillus niger* is highly cosmopolitan due to its wide range of temperature and pH tolerance (Perrone et al., 2007; Perfect et al., 2001).

A. niger is notorious for its saprophytic behavior in the soil. It is responsible for black mould disease in garlic, onion and shallot; root stalk rot of Sansevieria; and boll rot of cotton. This fungus causes spoilage of cashew dates, kernels, figs, and dried prune. Groundnut crown rot disease is also caused by *A. niger* (Bobbarala et al., 2009; Panchal and Dhale, 2011). These diseases that infest stored food end up in the gut of human and animals where it manifests several illnesses.

Controlling fungi species that cause havoc in stored food have its own controversies. Chemical pesticides usage is a popular way to control various plant diseases as compared to the natural means which deals with extracts from plants or plant parts.

Consumers are now much concern about the level of synthetic fungicides, because of their non-biodegradability

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nature. Many also argue that they are unfriendly to nature as they may affect untargeted beneficial organisms such as *Rhizobium* species which are natural nitrogen fixers. Several studies have revealed that plant extracts can be an alternative means of controlling fungi as natural pesticides (Arokiyaraj et al., 2008; Gangadevi et al., 2008; Brindha et al., 2009).

Ogo-Oluwa and Kator (2016) conclude from their research findings that *Vernonia amygdalina* possess inhibitory effect on rot causing fungi of tomato in storage and suggest it could also extend shelf life of the product. Research findings credited to Subbarao and Anna (2015) reveal that, neem supplements treated with manure is an inexpensive way to prevent pre-harvest contamination of EcO157 as they established that supplementing neem leaf and bark to manure resulted in elimination of pathogenic EcO157 in less than 10 days.

V. amygdalina, a member of the Asteraceae family*,* is a small shrub that grows in tropical Africa to a height of 2 to 5 m (6.6-16.4 ft). The leaves are elliptical and up to 20 cm (7.9 inch) long (Igile et al., 1995). The leaves are dark fleshy and green colored with a characteristic odor and a bitter taste. The species is indigenous to tropical Africa and is found wild or cultivated all over sub-Saharan Africa. The leaves are eaten, after crushing and washing thoroughly to remove the bitterness. All parts of the plant are pharmacologically useful. The cooked leaves are a staple vegetable in stews and soups of various cultures throughout equatorial Africa (Mayhew and Penny, 1998). *V. amygdalina* (Bitter leaf) is consumed either as a vegetable for cooking African dishes or the aqueous extract could be drank as tonics for the treatment of various ailments (Kigigha and Ebubechukwu, 2015; Imaga and Banigbetan, 2013). The bitterness is realized to be due to factors such as the presence of alkaloids, saponins, tannins and glycosides which have been shown by various authors to be present in bitter leaf (Butler and Bailey, 1973; Bonsi et al., 1995). Kambizi and Afolayan (2001) reported that acetone extract of *V. amygdalina* possesses antibacterial activity towards *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, *Micrococcus kristinae*, *Staphylococcus aureus*, *Enterobacter cloacae* and *Escherichia coli*.

Azadirachta indica (neem) is used in traditional medicine as a source of many therapeutic agents in the Indian culture and dwells well in the tropical countries. Its twigs provide a chewing stick and are wildly used in the Indian sub-continent (Almas and Ansal-Lafi, 1995). More than 135 compounds have been isolated from different parts of the tree. They have been divided into isoprenoid and non-isoprenoid compounds (Kumar and Parmar, 1996*)*. Neem has been used to control insect pest and diseases, as chewing stick and the water believed to be used as tongue cleaner (Birgit, 2013). Products of neem trees have been used in India for over two millennia for their medicinal properties, neem leaves have also been used to treat skin diseases like eczema and psoriasis (Anna, 2006). Moreover, the oil's antifungal, antibacterial, moisturizing and soothing potential permit its usage (Birgit, 2013).

This research work focused on assessing the best extract concentration of *A. indica* and *V. amygdalina* that is potent enough to curb the development of *A. niger* as a mycotoxin producing species.

MATERIALS AND METHODS

Collection of plant and test organism

A. indica and *V. amygdalina* were collected from the farms within Navrongo, Kassena Nankana Municipality in Upper East Region of Ghana. The pathogenic strain ATCC 16404 of *A. niger* was obtained from Food Research Institute Laboratory, Accra.

Extract preparation

Two separate extracts were prepared as the following.

Preparation of hot aqueous extract

Fresh leaves of plant samples were thoroughly washed under running tap. The leaves were then air-dried for two days. The dried leaves were blended into powder. Powdered samples (5, 4, 3, 2, and 1 g) were weighed separately into five volumetric flasks each containing 10 ml of sterile water. Concentration of 500, 400, 300, 200 and 100 mg/ml were made. The test tubes were then placed in water bath and heated for 1 h at 80°C in order not over heat the extract. The extracts were left overnight after which they were filtered into sterile test tubes using Whatman No. 1 filter paper (110 mm). The residue obtained was kept inside aluminum foil and the extracts were stored in refrigerator at a very low temperature (4°C) before use.

Preparation of ethanolic extract

Powdered samples (5, 4, 3, 2, and 1 g) were weighed separately into five test tubes each containing 10 ml of 75% ethanol which yielded respective concentrations of 500, 400, 300, 200, and 100 mg/ml. This was done to obtain various concentrations which will inhibit the fungi. The extracts were left over night after which they were filtered into sterile test tubes using Whatman No. 1 filter paper (110 mm). The filtrate obtained was evaporated to dryness at 45°C and the residue obtained was kept inside aluminium foil. The extracts were stored in refrigerator at a very low temperature (4°C) before use

Preparation of griseofulvin (Control)

Powdered Griseofulvin (5, 4, 3, 2, and 1 g) were weighed separately into five sterile test tubes each containing 10 ml of sterile distilled water which yielded concentrations of 500, 400, 300, 200 and 100 mg/ml.

Media preparation

Potato Dextrose salt agar (39 g) was dissolved in 1 ml of distilled

Table 1. Antifungal activity of dry *Azadirachta indica* leaves on *Aspergillus niger.*

Concentration levels (mg/ml)	Zones of inhibition (mm)		
	Hot aqueous extract of leaf	Ethanol extract of leaf	Control (Griseofulvin)
500	18.06±0.20	20.30±0.53	24.73±1.10
400	14.23±0.31	17.23±5.72	20.20±0.53
300	11.10±0.68	15.40±0.50	16.97±0.42
200	8.07±0.50	10.06±0.43	13.00±0.61
100	-	8.16±0.31	11.00±0.36

Table 2. Antifungal activity of dry *Vernonia amygdalina* leaves on *Aspergillus niger*.

Concentration levels (mg/ml)	Zones of inhibition (mm)		
	Hot aqueous extract of leaf	Ethanol extract of leaf	Control (Griseofulvin)
500	19.00±0.10	21.87±0.35	24.73±1.10
400	16.10±1.63	18.03±0.10	20.20±0.531
300	11.37±0.47	13.73±0.38	16.97±0.42
200	9.16±0.38	10.43±0.78	13.00±0.6
100	-	-	11.00±0.36

water. The mixture was stirred using magnetic stirrer and autoclaved at 121°C for 15 min. The media was cooled to 45°C to eliminate bubbles. 1 ml of 100 mg of streptomycin solution was prepared and added to the media to selectively inhibit the growth of bacterial cells. The prepared media was poured into the Petri dishes under sterile condition of lamella flow chamber to avoid contamination. Bubbles were eliminated from the poured media by flaming using Bunsen burner.

Inoculation of test organism (*A. niger*)

The test organism (*A. niger*) was inoculated using sterile inoculating loop under aseptic conditions. The loop was used to touch the freshly grown spores and transferred onto Petri plate containing sterile PDA. The test organism was streaked on the plate to ensure uniform spreading on the Petri plates.

Antimicrobial sensitivity test (Agar Well Diffusion Test)

A cork borer (6 mm in diameter) was sterilized by flaming and used to create four wells on each of the inoculated plates. The holes created were then filled with the plant extracts of different concentrations (100, 200, 300, 400 and 500 mg/ml). The plates were allowed to stand for 1 h for pre-diffusion of the extract (Esimone et al., 1998) and incubated at 25°C for 3 days. At the end of the incubation period, the plates were examined for the measurement of zone of inhibition (Hugo and Russel, 1996). The same sizes of holes were bored in which the control solutions of different concentration were incorporated. After a period of 3 days, the plates were examined and zones of inhibitions were measured by taking the distance within the zone across the well.

RESULTS

Antifungal activity of dry *A. indica* leaves on *A. niger*

The test organism was susceptible to the antifungal-control (Griseofulvin) at all the concentration levels. The first concentration showed a wider zone of inhibition (24.73 mm), followed by 20.20, 16.97, 13.00 and 11.00 mm at concentrations 500, 400, 300, 200 and 100 mg/ml, respectively. *A. niger* was also susceptible to ethanol extract at all the concentration levels, that is, 500, 400, 300, 200 and 100 mg/ml which gave inhibition zones of 20.30,17.23,15.40,10.06 and 8.16 mm, respectively. The aqueous extract exhibited the least zones of inhibition. *A. niger* were resistant at 100 mg/ml concentration (no inhibition zone was measured). Concentrations 500, 400, 300, and 200 mg/ml revealed inhibition zones of 18.06, 14.23, 11.10 and 8.07 mm, respectively shown in Table 1.

Antifungal activity of dry *V. amygdalina* leaves on *A. niger*

Table 2 shows the antifungal activity of *V. amgdalina* (Bitter leaf) extracts against *A. niger*. The test organism was susceptible to the antifungal (Griseofulvin) at all the concentration levels. The first concentration showed a wider zone of inhibition, that is, 24.73 mm, followed by 20.20, 16.97, 13.00 and 11.00 mm at concentrations of 500, 400, 300, 200 and 100 mg/ml, respectively. *A. niger* was also susceptible to ethanol extract at all the concentration levels, that is, 500, 400, 300, and 200 mg/ml, excluding 100 mg/ml which gave inhibition zones of 21.87, 18.03, 13.73, and 10.43 mm and no inhibition zone, respectively. The aqueous extract exhibited the least zones of inhibition and moreover, *A. niger* was resistant at 100 mg/ml concentration without an inhibition zone. Concentrations of 500, 400, 300, and 200 mg/ml

have shown inhibition zones of 19.0, 16.10, 11.37 and 9.16 mm, respectively.

DISCUSSION

The zones of inhibition showed that ethanolic extraction of both plants resources is superior to hot aqueous extraction in keeping *A. niger* inactive to proliferate. This finding is collaborated by research conclusions drawn by Susmith et al. (2013), Imaga et al. (2013) and Akah and Okafor (1992) who found neem and bitter leaves extract to contain alkaloids, tannins, flavonoids, steroids and saponins among others. These are also the active ingredients in plants that exert antimicrobial activity through different mechanisms (Igbinosa et al., 2009).

These phytoconstituents alkaloids, flavanoids, glycosides, and saponins act as antifungal principles of plants. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These phytochemical compounds exert antimicrobial activity through various mechanisms and the secondary metabolites in them are known to be biologically active and therefore play significant roles in bioactivity of medicinal plants because the medicinal values of medicinal plant lies in these phytochemical compounds which produced a definite and specific action on the human body (Kam and Liew, 2002). These phytochemical compounds are actually the defensive mechanism of the plants against different pathogens (Hafiza, 2000; Faiza aslam et al., 2009).

High concentration of the plant extracts could confer greater inhibitory effect from both extraction methods as evident in the results. This could be due the presence of much of several alkaloids efficiently resisting the growth of the fungi. Lower concentration of the extracts will also result in lesser inhibitory effect. This was also observed in the report by Esimone et al. (1998) which says that extract of plants inhibit the growth of various microorganisms at different concentrations. This was same as the results recorded in the present study where the increase in the concentration of extracts corresponds with the increase of the diameter of inhibition zones.

Conclusion

From the study, it could be concluded that both hot aqueous and ethanol extraction of the plants conferred inhibitory effect, but ethanolic extract yielded a greater effect of inhibition. The control had a greater zone of inhibition than both plants extracts.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Akah PA, Okafor CI (1992). "Blood sugar lowering effect of *Vernonia amygdalina Del*, in an experimental rabbit model." Phytotherapy Research 6(3):171-173.

Almas K, Ansal-Lafi TR (1995). The natural toothbrush. In World health forum. Geneva: World Health Organization 1980-1998. 16(2):206-211

Anna HP (2006). Neem Indian's tree of life. *BBC News* http://news.bbc.co.uk/2/hi/south_asia/4916044.stm

Arokiyaraj S, Martin S, Perinbam K, Marie AP, Beatrice V (2008). Free radical scavenging activity and HPTLC fingerprint of Pterocarpus santalinus L.-an in vitro study. Indian Journal of Science and Technology 1(7):1-3.

Birgit B (2013). Make neem tooth brush (Neem tree home remedies)-Discover neem. Retrieved July 2013, from https://www.discoverneem.com/neem-toothbrush.html

Bobbarala V, Katikala PK, Naidu KC, Penumajji S (2009). Antifungal activity of selected plant extracts against phytopathogenic fungi Aspergillus niger F2723. Indian Journal of Science and Technology 2(4):87-90.

Bonsi MK, Osuji PO, Tuah AK, Umuna MN (1995). *Vernonia amygdalina* as a supplement to teff straw (Eragrostis tef) fed to Ethiopian Menz sheep. Agroforestry systems 31(3):229-241.

Brindha V, Saravanan A, Manimekalai R (2009). Drug designing for ring finger protein 110 involved in adenocarcinoma (human breast cancer) using casuarinin extracted from *Terminalia arjuna*. Indian Journal of Science and Technology 2(2):22-26.

Butler GW, Bailey RW (1973). Chemistry and Biochemistry of Herbage.Vol.1 Accident Press, London and New York. https://www.journals.uchicago.edu/doi/abs/10.1086/408632

Esimone CO, Adikwu MU, Okonta, JM (1998). Preliminary antimicrobial screening of the ethanolic extract from the lichen Usnea subfloridans (L). Journal Pharmaceutical Research Development 3(2):99-101.

Faiza A, Khalil U, Rehman MA, Muhammed S (2009). Antibacterial activity of various phytoconstituents of Neem. Pakistan Journal of Agricultural Sciences 46(3):209-213.

Gangadevi V, Yogeswari S, Kamalraj S, Rani G, Muthumary J (2008). The antibacterial activity of Acalypha indica L. Indian journal of Science and Technology1(6). http://www.indjst.org/index.php/indjst/article/view/29582

Hafiza RE (2000). Peptides antibiotics. Lancet 349:418-422.

Igbinosa OO, Igbinosa EO, Aiyegoro OA (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). African journal of pharmacy and pharmacology 3(2):058-062.

Igile GO, Olesezk W, Burda S, Jurzysta M (1995). Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. Journal of Agricultural and Food Chemistry 43(8):2162-2166.

Imaga NOA, Banigbetan DO (2013). In vivo biochemical assessment of aqueous extracts of *Vernonia amygdalina* (Bitter leaf). International Journal of Nutrition and Metabolism 5(2):22-27.

Kam PCA, Liew A (2002). Traditional Chinese herbal medicine and anaesthesia. Anaesthesia 57(11):1083-1089.

Kambizi L, Afolayan AJ (2001). An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. Journal of ethnopharmacology 77(1):5-9.

Kigigha LT, Ebubechukwu O (2015). Antibacterial activity of bitter leaf (*Vernonia amygdalina*) soup on *Staphylococcus aureus* and *Escherichia coli*. Sky Journal of Microbiology Research 3(4):041-045.

Kumar J, Parmar BS (1996). Physicochemical and chemical variation in neem oils and some bioactivity leads against Spodoptera litura F. Journal of agricultural and food chemistry 44(8):2137-2143.

Mayhew S, Penny A (1998). Macmillian Tropical and Subtropical Foods. London: Macmillian Publishers. http://www.sciepub.com/reference/203704

Ogo-Oluwa AT, Kator L (2016). Evaluation of bitter leaf (*Vernonia amygdalina*) extract in the inhibition of fungi causing post harvest rot of tomato fruits in Makurdi, Benue State, Nigeria. Der Pharmacia Lettre 8(11):69-73 https://www.scholarsresearchlibrary.com/articles/evaluation-of-bitter-leaf-vernonia-amygdalina-extract-in-the-inhibition-of-fungi-causing-post-harvest-rot-of-tomato-frui.pdf

Panchal VH, Dhale DA (2011). Isolation of seed-borne fungi of sorghum (*Sorghum vulgare* pers.), Journal of Phytology 3(12):45-48.

Perfect ER, Cox GM, Lee JY, Chapman SW, Kauffman (2001). The impact of culture isolation of *Aspergillus* species: a hospital-based survey of aspergillosis. Clinical Infectious Diseases 33(11):1824-1833.

Perrone G, Susca A, Cozzi G, Ehrlich K, Varga J, Frisvad JC, Meijer M, Noonim P, Mahakarnchanakul W, Samson RA (2007). Biodiversity of *Aspergillus* species in some important agricultural products. Studies in Mycology 59:53-66.

Subbarao O, Anna K (2015). Effect of neem (Azadirachta indica) on the survival of Escherichia coli O157: H7 in dairy manure. International journal of environmental research and public health 12(7):7794-7803.

Susmitha S, Vidymol KK, Rangana-yaki P, Vijayaragavan R (2013). Phytochemical extraction and antimicrobial properties of *Azadirachta indica (Neem).* Global Journal of Pharmacology 7(3):316-320.

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Full Length Research Paper

Biotechnological potential of the *Carapa guianensis*, *Bertholletia excelsa* and *Copaifera* spp. oils

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The use of vegetable species for treatment or prevention of diseases is one of the most ancient medicine techniques in humanity. In Amazon rainforest, there are numerous of plant species that possess biotechnological potential, and due to this, this study aimed at carrying out a bibliographic review describing the biotechnological potential in *Carapa guianensis*, *Bertholletia excels*, and *Copaifera* spp. oils. This study is a systematic review of literature in the databases: SCIELO, VHL and PUBMED, by using the descriptors: *C. guianensis* oil, *B. excelsa* oil and *Copaifera* spp. After the selection, 87 articles were selected; 48 on *C. guianensis,* 9 on *B. excels*, and 30 on *Copaifera* spp. Oil seeds from Amazon rainforest possess a good biotechnological potential to be explored. This review has shown that *C. guianensis* and *Copaifera* spp. are strong candidates for the search of new insecticide, antiparasitict, anti-inflammatory and healing products. *Copaifera* spp. has also shown to be promising for the production of antibiotic and antifungic medicines, and *C. guianensis* for acaricide drugs. Future studies are indicated to deepen the knowledge already described and to investigate new biotechnological potentialities of Amazonian oil seeds. However, for this to happen, greater financial support is required from the Brazilian government and agencies, thus increasing the level of research in the region, which will consequently maximize the likelihood of new product discovery.

Key word: Biotechnology, oilseeds, Amazon rainforest.

INTRODUCTION

Ethnopharmacology is defined as “the interdisciplinary scientific exploration of biologically active agents

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Table 1. Amount of articles found in literature search.

Descriptor	SCIELO	VHL	PUBMED	Total
Carapa guianensis oil	26	52	30	108
Bertholletia excelsa oil	12	24	12	48
Copaifera spp oil[#]	23	64	12	99
Total	61	140	54	255

[#]Species searched: *Copaifera duckei* oil, *Copaifera langsdorffii* oil, *Copaifera martii* oil, *Copaifera paupera* oil, *Copaifera pubiflora* oil, and *Copaifera reticulata* oil.

traditionally employed or observed by man" (Bruhn and Holmstedt, 1981; Elisabetsky, 2003). This area combines information gained from medicinal flora users (communities and traditional specialists) with chemical and pharmacological studies (Elisabetsky, 2003).

The use of vegetable species for treatment and prevention of diseases is one of the most ancient medicine techniques in humanity (Dutra, 2009). Even though plants have been used for centuries for medicinal purposes, the vast majority have unknown biotechnological features by science (Meneguetti and Facundo, 2015).

Data show that about 95% of Brazilian native species still do not possess scientific studies on their possible applicabilities (Santos, 2009), representing a huge economic and biotechnological potential to be explored, especially in vegetable species from Amazon rainforest, as many have still not been catalogued by science (Cechinel and Rosendo, 1998; Abreu et al., 2001; Meneguetti and Facundo, 2015). It can present new chemical compounds and consequently new specific and efficient drugs (Lang et al., 2008).

There are plenty of plants species in Amazonian rainforest that possess biotechnological potential (Osakada, 2009), among them are presented *Carapa guianensis* (Andiroba), *Bertholletia excelsa* (Amazônia's nut) and *Copaifera* spp. (Copaíba). Furthermore, this study aimed at carrying out a bibliographic review describing the biotechnological potential of these species' oil.

MATERIALS AND METHODS

This study is a systematic review of literature based on Galvão and Pereira (2014), following the steps below:

(a) Creation of research question: What is the biotechnological potential described in literature of Andiroba, Amazônia's Nut, and Copaíba oils?
(b) Literature search: Search for scientific articles in the following databases: Scientific Electronic Library Online (SCIELO), Virtual Health Library (VHL) and National Institute of Health (PUBMED) by using the descriptors: *C. guianensis* oil, *B. excelsa* oil and *Copaifera* spp. oil of species (*Copaifera duckei* oil, *Copaifera langsdorffii* oil, *Copaifera martii* oil, *Copaifera paupera* oil, *Copaifera pubiflora* oil and *Copaifera reticulata* oil) because they were the main copaíba species in the Brazilian Amazon rainforest. Articles in Portuguese, English and Spanish published by 2018 were included. The amount of articles found in the survey is presented in Table 1.
(c) Article selection: This selection was performed by three researchers, and the articles rejected by two or three of them were removed from this research. The criteria for exclusion were repeated articles (found in more than one database), out of the research's aim, low methodological quality and with negative results for biotechnological potential.
d) Data gathering: After the selection, 87 articles were selected; 48 on *C. guianensis,* 9 on *B. excels*, and 30 on *Copaifera* spp. Those were used in the results of this study. Besides the selected articles, others were used for creation of introduction and improvement of this article's discussion.
e) Data synthesis: The data were organized into tables, and described in the text according to their biotechnological potential.
f) Results discussion writting: Description and discussion of the data are at "Results and Discussion" present in the sequence.

RESULTS AND DISCUSSION

Insecticide activity

The data showing that the oils searched have insecticide activity are shown in Table 2.

The *C. guianensis* and *Copaifera* spp. oils have shown a good potential in the medical entomology for vector control of tropical diseases, such as *A. aegypti*: Dengue, Chikungunya, and Zika virus vector (Aragão et al., 2018); *A. albopictus*: Yellow fever vector (IEC, 2018); and *C. quinquefasciatus*: that has been also found infected by Zika virus (Smartt et al., 2018).

C. guianensis develop products against ectoparasitosis due to their activity against *F. subrostratus* (popularly known as lice) that is a public health problem in Brazil. It is estimated that up to two thirds of deprived population and communities were affected in the beginning of the year 2000 (Heukelbach et al., 2003).

In livestock, *C. guianensis* has proven to be promising against two flies species: *M. domestica* and *H. irritans*, with this last one known popularly as "Horn Fly", which causes several problems in the Brazilian cattle (Klauck et al., 2014).

C. guianensis and *Copaifera* spp. may also be beneficial to agriculture, acting as anti-*S. frugiperda* popularly known as "Armyworm" (Santos et al., 2016), *A.*

Table 2. Insecticide oilseeds performance from Amazon rainforest.

Insect	*C. guianensis*	*B. excelsa*	*Copaifera* spp	Reference
Aedes aegypti (larvicide)	X	-	X	Mendonça et al. (2005)* Silva et al. (2006)* Abed et al. (2007)*** Silva et al. (2007)*** Geris et al. (2008)*** Rodrigues et al. (2014)***
Aedes aegypti (repellent)	X	-	-	Miot et al. (2004)* Jesus et al. (2017)*
Aedes albopictus (larvicide)	X	-	-	Silva et al. (2004)*
Atta sexdens rubropilosa	X	-	-	Ambrozin et al. (2006)*
Culex quinquefasciatus (larvicide)	-	-	X	Silva et al. (2003)***
Diabrotica speciosa	-	-	X	Barbosa et al. (2013)***
Felicola subrostratus	X	-	-	Barros et al. (2012)*
Haematobia irritans	X	-	-	Klauck et al. (2014)*
Musca domestica	X	-	-	Klauck et al. (2014)*
Spodoptera frugiperda	X	-	X	Santos et al. (2016)* Santos et al. (2016)***
Tribolium castaneum	-	-	X	Melo et al. (2015)***
Zabrotes subfasciatus	-	-	X	França et al. (2012)***

*Citations reference to *C. guianensis*; ** Citations referent to *B. excelsa*; *** Citations referent to Copaifera spp.

sexdens rubropilosa or “Sauba Ant” (Ambrozin et al., 2006), *D. speciosa* known as “vaquinha verde” (Barbosa et al., 2013), *Z. subfasciatus* known as “caruncho do feijão” (França et al., 2012) and *T. castaneum* as “May Beetle” (Melo et al., 2015), and they may cause jeopardy to diferrent types of crops.

In the databases, there were no articles found showing that *B. excels* has insecticide activity.

Acaricide activity

The *C. guianensis* oil was the most promising in this study; shown to be anti-*Anocentor nitens* (Farias et al., 2009; Farias et al., 2012)*, Rhipicephalus sanguineus* (Farias et al., 2009; Farias et al., 2012; Vendramini et al., 2012a; Vendramini et al., 2012b; Roma et al., 2013; Roma et al., 2015)*,* and *Rhipicephalus* (*Boophilus) microplus* (Farias et al., 2012; Chagas et al., 2012). The *B. excelsa* oil has also shown to be anti-*R. (B.) microplus*, however it was considered low (Villarreal et al., 2017).

The *A. nitens* tick is one of the species that affects horses, and it is vector of *Babesia caballi* protozoan, which is an etiologic agent of equine babesiosis, disease that promotes low development and death in animals (Borges and Leite, 1993; Bello et al., 2008). It is known as “Spinose Ear Tick”, and it is responsible for injuries in the pinna, productivity drop, irritation, blood spoliation, propensity to myiasis and secondary bacterial infections (Borges and Leite, 1993; Bello et al., 2008).

R. sanguineus has high prevalence in urban dogs, and is one of the species of parasites of these animals (Labruna and Pereira, 2001; Szabó et al., 2001; Soares et al., 2006; Paz et al., 2008). It is one responsible for pathogenic agent’s transmission, such as *Babesia canis* and *Eherlichia canis* (Smith et al., 1976; Gothe et al., 1989). There are records of infestation of this species in humans (Guglielmone et al., 2006), as they may become a secondary vector of Ehrlichiosis, Babesiosis and Spotted Fever (Fernandes et al., 2001; Paz et al., 2008).

R. (B.) microplus is the main species of ticks that affects cattle in Brazil, which causes reduction in milk production; the main economic impact due to it (Rodrigues and Leite, 2013). It may also cause jeopardy in meat and leather production, besides of several other diseases that can be transmitted by them (Massard and Fonseca, 2004; Andreotti et al., 2011; Santos et al., 2018).

Antiparasitic activity

Among the oils studied, parasitic activity was found against eight parasites species (Table 3).

The *C. guianensis* oil has shown activity against three species of goats and sheep gastronintestinal nematodes:

Table 3. Antiparasitic activity of Amazonian oleaginous oils.

Parasite	*C. guianensis*	*B. excelsa*	*Copaifera* spp.	Reference
Haemonchus sp.	X	-	-	Farias et al. (2010)*
Leishmania amazonenses	-	-	X	Santos et al. (2008a)*** Meneguetti et al. (2015)***
Leishmania chagasi	-	-	X	Rondon et al. (2012)*** Meneguetti et al. (2015)***
Oesophagostomum sp.	X	-	-	Farias et al. (2010)*
Plasmodium falciparum	X	-	-	Miranda juúnior et al. (2012)* Pereira et al. (2014)* Nardi et al. (2016)*
Plasmodium berghei	X	-	-	Pereira et al. (2014)*
Trichostrongylus sp	X	-	-	Farias et al. (2010)*
Trypanosoma evansi	-	-	X	Dorneles et al. (2013

*Citations reference to *C. guianensis*; ** Citations referent to *B. excelsa*; *** Citations referent to Copaifera spp.

Haemonchus sp., *Oesophagostomum* sp., and *Trichostrongylus* sp. (Farias et al., 2010). It has positive performance in veterinary and potential for future studies with helminths that affect humans.

C. guianensis was also antiparasitic against two species of *Plasmodium* gender, being the etiologic agent of malaria; disease among the four main epidemias in Latin America, with about 100 thousand new cases each year (Braz et al., 2006; Ferreira et al., 2012; Meneguetti et al., 2014). The anti-malaria potential is important because there is a need to search for new drugs against malaria, as it is resistant to nowadays drugs used, and being a threat to the disease control (Meneguetti et al., 2014).

Copaifera spp. has shown activity against trypanosomatids genders: *Leishmania* and *Trypanosoma,* Leishmaniasis and Chagas´ disease etiologic agents, diseases included in the group of Neglected Diseases, in which only 10% of the world's expenses with research in health are destined to diseases that account for 90% of the global illed people (Bezerra et al., 2012).

Leishmaniasis has shown the need for urgente new candidates for treatment drugs (Santos et al., 2013a), because today the first choice for leishmaniasis treatment have been pentavalent antinomials (Sb^{5+}), amidines, polyene, aminoglycosides, and hexadecylphosphocholine (miltefosine). However, some present toxicity for the patient (Bezerra et al., 2004; Figueredo et al., 2014; Meneguetti et al., 2015).

Currently the treatment of Chagas' disease presents only one drug available in Brazil, benzonidazole (2-nitroimidazole) introduced in the therapy in 1967 and to date a drug with satisfactory cure potential has not been developed and the drug used is inefficient and presents various side effects (Bezerra et al., 2012).

In the searched databases no articles were found that demonstrate the antiparasitic action of *B. excels*.

Antimicrobian activity

The oils studied showed antimicrobial activity against bacteria and fungi, as can be observed in Table 4. *Copaifera* spp. presented antimicrobial action; presented activity against 14 species, followed by *C. guianensis* that had action against 3 species. In the searched databases, no articles were found that demonstrate the antimicrobial action of *B. excelsa*.

The action against the microorganisms observed in Table 4, demonstrates the potential of the *Copaifera* spp. and *C. guianensis* oils for the development of drugs for the treatment of periodontal diseases, since it has had action against *A. actinomycetemcomitans, E. faecalis* and *P. gingivalis*. When they are present, several periodontal diseases are associated with cases of failure of endodontic treatment (Gasparetto et al., 2000; Carvalho and Cabral, 2007; Nacif and Alves, 2010).

Microbicidal activity against *E. coli*, *S. aureus*, *S. epidermidis* and *L. monocytogenes* is also highlighted, as they may cause several problems to human health, especially infections (Farbe and Peterkin, 1991; Lowy, 1998; Otto, 2009; Matos et al., 2015), which are often resistant to antibiotics (Santos, 2014), which demonstrates the importance of research for the development of new drugs.

The *Copaifera* spp. oil has an antifungal potential, with action against the dermatophyte *T. rubrum* (Dias et al., 2015) and species of the genus *Candida*, which causes the disease candidiasis (Calderone and Fonzi, 2001), which according to the *Associação de Obstetrícia e Ginecologia do Estado de São Paulo* (SOGESP - Association of Obstetrics and Gynecology of São Paulo State), affects 75% of women and of these, almost half will have a second episode and about 5% will present the condition more than once a year (Galileu, 2018). There is a large pharmacological market to be exploited for the

Table 4. Antimicrobial activity of Amazonian oleaginous oils.

Microrganism	*C. guianensis*	*B. excelsa*	*Copaifera* spp.	Reference
Actinobacillus actinomycetemcomitans	-	-	X	Dias et al. (2015)***
Bacillus cereus	-	-	X	Santos et al. (2013a)***
Bacillus subtilis	-	-	X	Santos et al. (2008b)***
Candida glabrata	-	-	X	Alencar et al. (2015)***
Candida krusei	-	-	X	Alencar et al. (2015)***
Enterococcus faecalis	X	-	X	Meccia et al. (2013)* Santos et al. (2008b)***
Escherichia coli	X	-	-	Brito et al. (2001)*
Listeria monocytogenes	-	-	X	Santos et al. (2013b)***
Microsporum canis	-	-	X	Dias et al. (2015)***
Porphyromonas gingivalis	-	-	X	Dias et al. (2015)***
Staphylococcus aureus	X	-	X	Brito et al. (2001)* Meccia et al. (2013)* Santos et al. (2008b)*** Santos et al. (2013a)*** Alencar et al. (2015)***
Staphylococcus epidermidis	-	-	X	Alencar et al. (2015)*** Santos et al. (2008b)***
Streptococcus mitis	-	-	X	Dias et al. (2015)***
Streptococcus sp	-	-	X	Ziech et al. (2013)***
Trichophyton rubrum	-	-	X	Dias et al. (2015)***

*Citations reference to *C. guianensis*; ** Citations referent to *B. excelsa*; *** Citations referent to *Copaifera* spp.

production of antifungal agents.

Other activities with biotechnological potential

Other activities of *C. guianensis*, *B. excelsa* and *Copaifera* spp. can be observed in Table 5.

The anti-inflammatory and healing effects of *C. guianensis* and *Copaifera* spp. are in agreement with their popular use, since both are used for these purposes, demonstrating the importance of the empirical knowledge of traditional populations for scientific research.

The antioxidant effect of *C. guianensis* and *B. excelsa* is very well seen in the pharmacological and food industry, since antioxidants prevent the formation of free radicals in the body, retarding cellular aging (Silva and Ferrari, 2011), which if added to the antigenotoxic effect of *C. guianensis* (Lemes et al., 2017) may be an alternative for future studies of cancer treatment. In the case of *B. excelsa*, which is used for the production of olive oils (Valdez et al., 2009) and other beverages (Felberg et al., 2009), the antioxidant characteristic, together with its nutritional power (Spini et al. 2006) further yields economic value to the product.

In addition to all the activities observed in Table 5, in a study with rats, *C. guianensis* did not produce toxic effects (Costa-Silva et al., 2008), genotoxic (Milhomem-Paixão et al., 2016), did not provoke chromosome aberrations in bone marrow cells (Arrebola et al., 2013) and did not interfere in the fertility and development of offspring (Costa-Silva et al., 2006). These data demonstrate a safety for the production of drugs from this species.

Copaifera spp. has also demonstrated safety for the use of oil as a therapeutic agent, since it does not present acute toxicity and neurotoxic effects (Sachetti et al., 2009) and still has neuroprotective action (Santos et al., 2012). When used in combination with vaginal cream and applied to rats, it has been shown to be safe during pregnancy (Lima et al., 2011).

B. excelsa has also been shown to be a safe species for use, since its almond is already widely used in food. This species still presents biodiesel production potential, as was observed in *C. guianensis* (Stachiw et al., 2016), which has also been shown to be a good catalyst (Tiosso et al., 2014).

Conclusion

It was verified that the Amazonian oilseeds have a good

Table 5. Other activities of Amazon rainforest oleaginous oils.

Activity	*C. guianensis*	*B. excelsa*	*Copaifera* spp	Reference
Antiallergic	X	-	-	Penido et al. (2005)* Nardi et al. (2016)* Ferraris et al. (2011)* Ferraris et al. (2012)* Henriques and Penido, 2014)*
Anti-inflammatory	X	-	X	Penido et al. (2005)* Penido et al. (2006)* Henriques and Penido, 2014)* Nardi et al. (2016)* HiguchI et al. (2017)* Wanzeler et al. (2018)* Carvalho et al. (2005)*** Muniz et al. (2009)*** Teixeira et al. (2017)***
Antioxidant	X	X	-	Milhomem-Paixão et al. (2016)* Vieira and Regitano-D'arce, 1999)*)* Gomes et al. (2016)*)*
Antigenotoxic	X	-	-	Lemes et al. (2017)*
Healing	X	-	X	Botelho-Brito et al. (2001)* Santos et al. (2013a)* Silva et al. (2015)* Wanzeler et al. (2018)* Estevão et al. (2009)*** Estevão et al. (2013)*** Feitosa Junior et al. (2018)***
Neuroprotective	-	-	X	Santos et al. (2012)***

biotechnological potential to be explored. The present review showed that *C. guianensis* and *Copaifera* spp. are strong candidates in searching for new products with insecticidal, antiparasitic, anti-inflammatory and cicatrizing activity.

Copaifera spp. has also been shown to be promising for the production of antibiotics and antifungals and *C. guianensis* for acaricidal drugs.

Future studies are indicated to deepen the knowledge already described and to investigate new biotechnological potentialities of Amazonian oilseeds. However, for this to happen, greater financial support is required from the Brazilian government and agencies and the interaction between different researchers, laboratories and research groups to form multidisciplinary and interdisciplinary teams, thus increasing the level of research in the region, which will consequently maximize the likelihood of new product discovery.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abed RAAA, Cavasin GM, Silva HHG, Geris RG, Silva IG (2007). Alterações morfohistológicas em larvas de *Aedes aegypti* (Linnaeus, 1762) (Diptera, Culicidae) causadas pela atividade larvicida do óleo-resina da planta medicinal *Copaifera reticulata* Ducke (Leguminosae). Revista De Patologia Tropical 36(1):75-86. https://doi.org/10.5216/rpt.v36i1.1819

Abreu AS, Barbosa OS, Müller AH, Guilhon GMSP (2001). Constituintes químicos do caule e das cascas do caule de C*roton pullei* var. Glabrior (Euphorbiaceae). Revista Virtual de Iniciação Acadêmica da UFPA 1(2):1-9.

Alencar EM, Xavier-Júnior FH, Morais AR, Dantas TR, Dantas-Santos N, Verissimo LM (2015). Chemical Characterization and Antimicrobial Activity Evaluation of Natural Oil Nanostructured Emulsions. Journal of Nanoscience and Nanotechnology 15(1):880-888. http://dx.doi.org/10.1166/jnn.2015.9187

Ambrozin ARP, Leite AC, Bueno FC, Vieira PC, Fernandes JB, Bueno OC (2006). Limonoids from andiroba oil and Cedrela fissilis and their insecticidal activity. Journal of the Brazilian Chemical Society 17(3):542-547.http://dx.doi.org/10.1590/S0103-50532006000300017

Andreotti R, Guerrero FD, Soares MA, Barros JC, Miller RJ, Léon AP (2011). Acaricide resistance of *Rhipicephalus (Boophilus) microplus* in State of Mato Grosso do Sul, Brazil. Revista Brasileira de Parasitologia Veterinária 20(2):127-133. http://dx.doi.org/10.1590/S1984-29612011000200007

Aragão CF, Cruz ACR, Neto JPN, Monteiro HAO, Silva EVP, Silva SP (2018). Circulation of Chikungunya virus in *Aedes aegypti* in

Maranhão, Northeast Brazil. Acta Tropica 15(1):880-888. http://dx.doi.org/10.1016/j.actatropica.2018.06.022
Barbosa FS, Leite GLD, Martins ER, D'avila VA, Cerqueira VM (2018). Medicinal plant extracts on the control of *Diabrotica speciosa* (Coleoptera: Chrysomelidae). Revista Brasileira de Plantas Medicinais 15(1):142-149. http://dx.doi.org/10.1590/S1516-05722013000100020
Barros FN, Farias MPO, Tavares JPC, Alves LC, Faustino MAG (2012). In vitro efficacy of oil from the seed of *Carapa guianensis* (andiroba) in the control of *Felicola subrostratus*. Revista Brasileira de Farmacognosia 22(5):1130-1133. http://dx.doi.org/10.1590/S0102-695X2012005000047
Bello AC, Cunha AP, Leite RC, Oliveira PR, Ribeiro AC, Domingues LN (2008). Controle de *Anocentor nitens* (Neumann, 1897) (Acari: Ixodidae) em Equinos. Revista Brasileira de Parasitologia Veterinária 17(1):59-63.
Bezerra RJS, Leon L, Genestra M (2004). Recentes avanços da quimioterapia das leishmanioses: moléculas intracelulares como alvo de fármacos. Revista Brasileira de Ciências Farmacêuticas 40(2):141-148. http://dx.doi.org/10.1590/S1516-93322004000200003
Bezerra WS, Meneguetti DUO, Camargo LMA (2012). A Busca de fármacos para tratamento da Tripanossomíase Americana: 103 anos de negligência. Saúde (Santa Maria) 38(1):09-20. http://dx.doi.org/10.5902/223658344813
Borges LMF, Leite RC (1993). Aspectos biológicos do *Dermacentor nitens* (Neumann, 1897) em condições de laboratório. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 45(6):586-591.
Botelho-Brito NM, Silva PRFS, Silva GCF, Casella SFM, Sampaio ARS, Carvalho RA (2001). Avaliação macroscópica de feridas cutâneas abertas, em ratos, tratadas com óleo de andiroba/Macroscopic evaluation of the cicatrization of treated open cutaneous wounds with oil andiroba in rats. Revista Paraense Medicina 15(2):17-22.
Braz RM, Andreozziii VL, Kale PL (2006). Detecção precoce de epidemias de malária no Brasil: uma proposta de automação. Epidemiologia e Serviços de Saúde 15(2):21-33. http://dx.doi.org/10.5123/S1679-49742006000200004
Brito MVH, Brazão RV, Siqueira RBP, Santos MT (2001). Efeito do óleo de andiroba em cultura de *Staphylococcus aureus* e *Escherichia coli*: estudo in vitro. Revista Paraense Medicina 15(1):36-40.
Bruhn JG, Holmstedt B (1981). Ethnopharmacology, objectives, principles and perspectives. In: Reinhard JLE. Natural products as medicinal agents. Stuttgart: Hippokrates, 1981.
Calderone RA, Fonzi WA (2001). Virulence factors of *Candida albicans*. Trends in Microbiology 9(7):327-335.
Carvalho C, Cabral CT (2007). Papel da *Porphyromonas Gingivalis* na Doença Periodontal. Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial 48(3):167-171. https://doi.org/10.1016/S1646-2890(07)70136-X
Carvalho JC, Cascon V, Possebon LS, Morimoto MS, Cardoso LG, Kaplan MA (2005). Topical antiinflammatory and analgesic activities of *Copaifera duckei* dwyer. Phytotherapy Research 19(11):946-950.
Cechinel FV, Rosendo AY (1998). Estratégias para a obtenção de compostos farmacologicamente ativos a partir de plantas medicinais. Conceitos sobre modificação estrutural para otimização da atividade. Química Nova 21(1):99-105. http://dx.doi.org/10.1590/S0100-40421998000100015
Chagas SAC, Barros LD, Cotinguiba F, Furlan M, Giglioti R, Sena Oliveira MC, Bizzo HR (2012). In vitro efficacy of plant extracts and synthesized substances on Rhipicephalus (Boophilus) Microplus (Acari: Ixodidae). Parasitology Research 110(1):295-303.
Costa-Silva JH, Lima CR, Silva EJ, Araújo AV, Fraga MC, Ribeiro A (2008). Acute and subacute toxicity of the *Carapa guianensis* Aublet (Meliaceae) seed oil. Journal of Ethnopharmacology 116(3):495-500. http://dx.doi.org/10.1016/j.jep.2007.12.016
Costa-Silva JH, Lyra MM, Lima CR, Arruda VM, Araújo AV, Ribeiro A (2007). A toxicological evaluation of the effect of *Carapa guianensis* Aublet on pregnancy in Wistar rats. Journal of Ethnopharmacology 112(1):122-126. http://dx.doi.org/10.1016/j.jep.2007.02.004
Dias FGG, Casemiro LA, Martins CHG, Dias LGGG, Pereira LF, Nishimura LT (2015). Endodontics pastes formulated with *copaiba oil*: action on oral microbiota and dentin bridge formation in dogs. Ciência Rural 45(6):1073-1078.
Dutra MG (2009). Plantas medicinais, fitoterápicos e saúde pública: um diagnóstico situacional em Anápolis, Goiás. Dissertação (Mestrado) - Centro Universitário de Anápolis, Anápolis - Goiás.
Elisabetsky E (2003). Etnofarmacologia. Ciencia e Cultura 55(3):35-36.
Estevão LRM, Medeiros JP, Baratella-Evêncio L, Simões RS, Mendonça FS, Evêncio-Neto J (2013). Effects of the topical administration of copaiba oil ointment (*Copaifera langsdorffii*) in skin flaps viability of rats. Acta Cirurgica Brasileira 28(12):863-869. http://dx.doi.org/10.1590/S0102-86502013001200009
Estevão LRM, Medeiros JP, Scognamillo-Szabó MVR, Baratella-Evêncio L, Guimarães EC, Câmara CAG, Evêncio-Neto J (2009). Neoangiogênese de retalhos cutâneos em ratos tratados com óleo de copaíba. Pesquisa Agropecuária Brasileira 44(4):406-412.
Farbe JM, Peterkin PI (1991). Listeria monocytogenes, a food-borne pathogen. Microbiological Reviews 55(3):476-511.
Farias MPO, Sousa DP, Arruda AC, Wanderley AG, Teixeira WC, Alves LC, Faustino MAG (2009). Potencial acaricida do óleo de andiroba *Carapa guianensis* Aubl. sobre fêmeas adultas ingurgitadas de *Anocentor nitens* Neumann, 1897 e *Rhipicephalus sanguineus* Latreille, 1806. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 61(4):877-882.
Farias MPO, Teixeira WC, Wanderley AG, Alves LC, Faustino MAG (2010). Avaliação in vitro dos efeitos do óleo da semente de Carapa guianensis Aubl. sobre larvas de nematóides gastrintestinais de caprinos e ovinos. Revista Brasileira de Plantas Medicinais 12(2):220-226.
Farias MPO, Wanderley AG, Alves LC, Faustino MAG (2012). Cálculo da CI50 (concentração inibitória média) e CL50 (concentração letal média) do óleo da semente de andiroba (*Carapa guianensis*, Aubl.) sobre *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887), *Anocentor nitens* (Neumann, 1897) e *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae). Arquivos do Instituto Biológico 79(2):255-261. http://dx.doi.org/10.1590/S1808-16572012000200014
Feitosa Junior DJS, Carvalho LTF, Rocha IRO, Brito CN, Moreira RA, Barros CAV (2018). Effects of Copaiba oil in the healing process of urinary bladder in rats. International Brazilian Journal of Urology 44(2):384-389. http://dx.doi.org/10.1590/s1677-5538.ibju.2017.0143
Felberg I, Antoniassi R, Deliza R, Freitas SC, Modesta RCD (2009). Soy and Brazil nut beverage: processing, composition, sensory, and color evaluation. Food Science and Technology 29(3):609-617. http://dx.doi.org/10.1590/S0101-20612009000300002
Fernandes FF, Freitas EP, Silva JR, Silva OR, Silva IG (2001). Efeitos toxicológicos e ineficiência in vitro de deltametrina sobre larvas de *Rhipicephalus sanguineus*, de Goiânia, Goiás, Brasil. Revista da Sociedade Brasileira de Medicina Tropical 34(2):159-165. http://dx.doi.org/10.1590/S0037-86822001000200002
Ferraris FK, Moret KH, Figueiredo AB, Penido CC, Henriques MD (2012). Gedunin, a natural tetranortriterpenoid, modulates T lymphocyte responses and ameliorates allergic inflammation. International Immunopharmacology 14(1):82–93. http://dx.doi.org/10.1016/j.intimp.2012.06.002
Ferraris FK, Rodrigues R, Silva VP, Figueiredo R, Penido C, Henriques MD (2011). Modulation of T lymphocyte and eosinophil functions in vitro by natural tetranortriterpenoids isolated from *Carapa guianensis* Aublet. International Immunopharmacology 11(1):1-11. http://dx.doi.org/10.1016/j.intimp.2010.09.010
Ferreira GM, Zan RA, Ramos LJ, Souza RAA, Meneguetti DUO (2012). Panorama Epidemiológico da Malária no Município de Ariquemes, Rondônia, Amazônia Ocidental: Um Inquérito de Seis Anos (2005 a 2010). Revista de Epidemiologia e Controle de Infecção 2(4):128-132. http://dx.doi.org/10.17058/reci.v2i4.27
Figueredo FG, Tintino SR, Brito DIV, Braga MFBM, Leite NF, Lucena BFF (2014). Avaliação das potenciais atividades tripanocida e antileishmania do extrato de folhas de *Piper arboreum* (Piperaceae) e de suas frações. Revista de Ciências Farmacêuticas Básica e Aplicada 35(1):149-154. http://dx.doi.org/10.1590/1983-084X/15_215
França SM, Oliveira JV, Esteves Filho AB, Oliveira CM (2012). Toxicity and repellency of essential oils to *Zabrotes subfasciatus* (Boheman) (Coleoptera, Chrysomelidae, Bruchinae) in *Phaseolus vulgaris* L. Acta Amazônica 42(3):381-386. http://dx.doi.org/10.1590/S0044-59672012000300010

Galileu V (2018). Candidíase: saiba tudo sobre a doença que afeta 75% das mulheres. Disponível em <https://revistagalileu.globo.com/Ciencia/Saude/noticia/2017/07/candidiase-saiba-tudo-sobre-doenca-que-afeta-75-das-mulheres.html>.

Galvão TF, Pereira MG (2014). Revisões sistemáticas da literatura: passos para sua elaboração. Epidemiologia e Serviços de Saúde 23(1):183-184. http://dx.doi.org/10.5123/S1679-49742014000100018

Gasparetto A, Arana-Chavez VE, Avila-Campos MJ (2000). Aderência de *Actinobacillus actinomycetemcomitans* às células epiteliais bucais: estabilidade e aspectos ultra-estruturais. Pesquisa Odontológica Brasileira 14(4):311-318. http://dx.doi.org/10.1590/S1517-74912000000400002

Geris R, Silva IG, Silva HH, Barison A, Rodrigues-Filho E, Ferreira AG (2008). Diterpenoids from *Copaifera reticulata* Ducke with larvicidal activity against *Aedes aegypti* (l.) (Diptera, culicidae). Revista do Instituto de Medicina Tropical de São Paulo 50(1):25-28. http://dx.doi.org/10.1590/S0036-46652008000100006

Gomes S, Torres AG (2016). Optimized extraction of polyphenolic antioxidant compounds from Brazil nut (*Bertholletia excelsa*) cake and evaluation of the polyphenol profile by HPLC. Journal of the Science of Food and Agriculture 96(8):2805-2814. http://dx.doi.org/10.1002/jsfa.7448

Gothe R, Wegerot S, Walden R, Walden A (1989). Epidemiology of *Babesia canis* and *Babesia gibsoni* infections in dogs in Germany. Kieintierpraxis 34(7):309-320.

Guglielmone AA, Beati L, Barros-Battesti DM, Labruna MB, Nava S, Venzal JM (2006). Ticks (Ixodidae) on humans in South America. Experimental and Applied Acarology 40(2):83-100. http://dx.doi.org/10.1007/s10493-006-9027-0

Henriques Md, Penido C (2014). The therapeutic properties of *Carapa guianensis*. Current Pharmaceutical Design 20(6):850-856. http://dx.doi.org/10.2174/13816128113199990048

Heukelbach J, Oliveira FAZ, Feldmeier H (2003). Ectoparasitoses e saúde pública no Brasil: desafios para controle. Cadernos de Saúde Pública 19(5):1535-1540. http://dx.doi.org/10.1590/S0102-311X2003000500032

Higuchi K, Miyake T, Ohmori S, Tani Y, Minoura K, Kikuchi T (2017). Carapanosins A-C from Seeds of Andiroba (*Carapa guianensis*, Meliaceae) and Their Effects on LPS-Activated NO Production. Molecules 22(3):e502. http://dx.doi.org/10.3390/molecules22030502

Instituto Evandro Chagas (IEC) (2018). Instituto Evandro Chagas detecta vírus da Febre Amarela em mosquito *Aedes albopictus* no Brasil. Disponível em <www.iec.gov.br/portal/descoberta/>.

Jesus FLM, Almeida FB, Duarte JL, Oliveira AEMFM, Cruz RAS, Souto RNP (2017). Preparation of a Nanoemulsion with *Carapa guianensis* Aublet (Meliaceae) Oil by a Low-Energy/Solvent-Free Method and Evaluation of Its Preliminary Residual Larvicidal Activity. Evidence-Based Complementary and Alternative Medicine 2017:e6756793. https://doi.org/10.1155/2017/6756793

Klauck V, Pazinato R, Stefani LM, Santos RC, Vaucher RA, Baldissera MD (2014). Insecticidal and repellent effects of tea tree and andiroba oils on flies associated with livestock. Medical and Veterinary Entomology 28(suppl.1):33-39. http://dx.doi.org/10.1111/mve.12078

Labruna MB, Pereira MC (2001). Carrapato em Cães no Brasil. Clínica Veterinária 30(1):24-32.

Lang G, Mayhundin NA, Mitova MI, Sun L, Van Der Sar S, Blunt JW (2008). Evolving trends in the dereplication of natural products extracts: New methodology for rapid, small-scale investigation of natural products. Journal of Natural Productsa 71(9):1595-1599. http://dx.doi.org/10.1021/np8002222

Lemes SR, Chaves DA, Júnior NJS, Carneiro CC, Chen-Chen L, Almeida LM, Gonçalves PJ, Melo-Reis PR (2017). Antigenotoxicity protection of Carapa guianensis oil against mitomycin C and cyclophosphamide in mouse bone marrow. Anais da Academia Brasileira de Ciências 89(3):2043-2051.

Lima CS, Medeiros BJ, Favacho HA, Santos KC, Oliveira BR, Taglialegna JC (2011). Pre-clinical validation of a vaginal cream containing copaiba oil (reproductive toxicology study). Phytomedicine 18(12):1013-1023. http://dx.doi.org/10.1016/j.phymed.2011.05.004

Lowy FD (1998). *Staphylococcus aureus* Infections. The New England Journal of Medicine 339:520-532.

Massard CL, Fonseca AH (2004). Carrapatos e doenças transmitidas, comuns ao homem e aos animais. A Hora Veterinária 135(1):15-23.

Matos BN, Pieri FA, Penatti M, Orlandi PP (2015). Adherence and virulence genes of *Escherichia coli* from children diarrhoea in the Brazilian Amazon. Brazilian Journal of Microbiology 46(1):131-137. http://dx.doi.org/10.1590/S1517-838246120130917

Meccia G, Quintero P, Rojas LB, Usubillaga A, Velasco J, Diaz T (2013). Chemical composition of the essential oil from the leaves of *Carapa guianensis* collected from Venezuelan Guayana and the antimicrobial activity of the oil and crude extracts. Natural Product Communications 8(11):1641-1642. http://dx.doi.org/10.1177/1934578x1300801135

Melo BA, Almeida FAC, Silva JF, Silva RM (2015). Atividade inseticida do óleo de *Copaifera langsdorffii* Desf. (copaiba) sobre *Tribolium castaneum* (Coleoptera: Tenebrionidae). Revista Cubana de Plantas Medicinales 20(4):419-428.

Mendonça FA, Silva KF, Santos KK, Ribeiro Júnior KA, Sant'ana AE (2005). Activities of some Brazilian plants against larvae of the mosquito *Aedes aegypti*. Fitoterapia 76(7-8):629-636. https://doi.org/10.1016/j.fitote.2005.06.013

Meneguetti DUO, Facundo VA (2015). Análise genotóxica e antiparasitária de extratos e substâncias isoladas de *Maytenus guianensis* Klotzsch ex Reissek (Celastraceae), Chichuá (Xixuá) amazônico. Revista Pan-Amazônica de Saúde 6(4):69-70. http://dx.doi.org/10.5123/S2176-62232015000400010

Meneguetti DUO, Cunha RM, Lima RA, Oliveira FAZ, Medeiros DSS, Passarini GM, Medeiros PSM, Militão SLT, Facundo VA (2014) Antimalarial ethnopharmacology in the Brazilian Amazon. Revista de Ciências Farmacêuticas Básica e Aplicada 35(4):577-587.

Meneguetti DUO, Lima RA, Macedo SRA, Barros NB, Facundo JB, Militao JSLT, Nicolete R, Facundo VA (2015). Plantas da Amazônia Brasileira com potencial leishmanicida *in vitro*. Revista de Patologia Tropical 44(4):359-374. http://dx.doi.org/10.5216/rpt.v44i4.39236

Milhomem-Paixão SSR, Fascineli ML, Roll MM, Longo JPF, Azevedo RB, Pieczarka JC (2016). The lipidome, genotoxicity, hematotoxicity and antioxidant properties of andiroba oil from the Brazilian Amazon. Genetics and Molecular Biology 39(2):248-256. http://dx.doi.org/10.1590/1678-4685-gmb-2015-0098

Miot HA, Batistella RF, Batista KA, Volpato DEC, Augusto LST, Madeira NG, Haddad-Jr V, Miot LDB (2004). Comparative study of the topical effectiveness of the Andiroba oil (*Carapa guianensis*) and DEET 50% as repellent for *Aede*s sp. Revista do Instituto de Medicina Tropical de São Paulo 46(5):253-256.

Muniz JWC, Bozza PT, Nascimento JLM, Reis PA (2009). Atividade Anti-Inflamatória do Óleo-Resina da *Copaífera Reticulata* em Modelo Inflamatório de Edema de Pata. Revista Paraense de Medicina 23:15-21. http://dx.doi.org/10.1590/0100-69912016006006

Nacif MCAM, Alves FRF (2010). *Enterococcus faecalis* na endodontia: um desafio ao sucesso. Revista Brasileira de Odontologia 67(2):209-214. http://dx.doi.org/10.18363/rbo.v67n2.p.209

Nardi M, Lira-Guedes AC, Cunha HFA, Guedes MC, Mustin K, Gomes SC (2016). Artisanal Extraction and Traditional Knowledge Associated with Medicinal Use of Crabwood Oil (Cara*pa guianensis* Aublet.) in a Peri-Urban Várzea Environment in the Amazon Estuary. Evidence-Based Complementary and Alternative Medicine 2016:e5828021. http://dx.doi.org/10.1155/2016/5828021

Osakada A (2009). Desenvolvimento inicial de sangue-de-dragão (*Croton lechleri* MULL. ARG) sob diferentes classes de solos, corretivos e níveis de luminosidade na Amazônia central. Dissertação (Mestrado) Instituto Nacional de Pesquisas da Amazônia, Manaus, - AM.

Otto M (2009). *Staphylococcus epidermidis* - the 'accidental' pathogen. Nature Reviews Microbiology 7(8):555–567. http://dx.doi.org/10.1038/nrmicro2182

Paz GF, Leite RC, Oliveira PR (2008). Controle de *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) no canil da Escola de Veterinária da UFMG, Belo Horizonte, Minas Gerais, Brasil. Revista Brasileira de Parasitologia Veterinária 17(1):41-44. http://dx.doi.org/10.1590/S1984-29612008000100009

Penido C, Costa KA, Pennaforte RJ, Costa MF, Pereira JF, Siani AC (2005). Anti-allergic effects of natural tetranortriterpenoids isolated from *Carapa guianensis* Aublet on allergen-induced vascular permeability and hyperalgesia. Inflammation Research 54(7):295-

303. http://dx.doi.org/10.1007/s00011-005-1357-6
Penido C, Conte FP, Chagas MS, Rodrigues CA, Pereira JF, Henriques MG (2006). Antiinflammatory effects of natural tetranortriterpenoids isolated from *Carapa guianensis* Aublet on zymosan-induced arthritis in mice. Inflammation Research 55(11):457-464. http://dx.doi.org/10.1007/s00011-006-5161-8
Rodrigues DS, Leite RC (2013). Impacto econômico de Rhipicephalus (Boophilus) microplus: estimativa de redução de produção de leite. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 65(5):1570-1572. http://dx.doi.org/10.1590/S0102-09352013000500039
Rodrigues ECR, Ferreira AM, Vilhena JCE, Almeida FB, Cruz RAS, Florentino AC, Souto RNP, Carvalho JCT, Fernandes CP (2014). Development of a larvicidal nanoemulsion with Copaiba (*Copaifera duckei*) oleoresin. Revista Brasileira de Farmacognosia 24(6):699-705.
Roma GC, Camargo-Mathias MI, Nunes PH, Remédio RN, Faria AU, Bechara GH (2015). Effects of andiroba (*Carapa guianensis*) oil in ticks: Ultrastructural analysis of the synganglion of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae). Acta Tropica 141(PtA):7-15. http://dx.doi.org/10.1016/j.actatropica.2014.06.018
Roma GC, Mathias MI, Faria AU, Oliveira PR, Furquim KC, Bechara GH (2013). Morphological and cytochemical changes in synganglion of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) female ticks from exposure of andiroba oil (*Carapa guianensis*). Microscopy Research and Technique 76(7):687-696. http://dx.doi.org/10.1002/jemt.22219
Rondon FCM, Bevilaqua CML, Accioly MP, Morais SM, Andrade-Júnior HF, Carvalho CA (2012). In vitro efficacy of *Coriandrum sativum*, *Lippia sidoides* and *Copaifera reticulata* against *Leishmania chagasi.* Revista Brasileira de Parasitologia Veterinária 21(3):185-191. http://dx.doi.org/10.1590/S1984-29612012000300002
Sachetti CG, Fascineli ML, Sampaio JA, Lameira OA, Caldas ED (2009). Avaliação da toxicidade aguda e potencial neurotóxico do óleo-resina de copaíba (*Copaifera reticulata* Ducke, Fabaceae). Revista Brasileira de Farmacognosia 19(4):937-941. http://dx.doi.org/10.1590/S0102-695X2009000600025
Santos ACV, Fernandes CC, Lopes LM, Sousa AH (2016). Inseticidal oils from amazon plants in control of fall armyworm. Revista Caatinga 29(3):642-647. http://dx.doi.org/10.1590/1983-21252016v29n314rc
Santos AO, Ueda-Nakamura T, Dias Filho BP, Veiga Junior VF, Pinto AC, Nakamura CV (2008a). Effect of Brazilian copaiba oils on *Leishmania amazonensis.* Journal of Ethnopharmacology 120(2):204-208. http://dx.doi.org/10.1016/j.jep.2008.08.007
Santos AO, Ueda-Nakamura T, Dias Filho BP, Veiga Junior VF, Pinto AC, Nakamura CV (2008b). Antimicrobial activity of Brazilian copaiba oils obtained from different species of the *Copaifera genus*. Memórias do Instituto Oswaldo Cruz 103(3):277-281. http://dx.doi.org/10.1590/S0074-02762008005000015
Santos ECG, Donnici CL, Camargos ER, Rezende AA, Andrade EH, Soares LA, Farias LM, Carvalho MAR, Almeida MG (2013a). Effects of Copaifera duckei Dwyer oleoresin on the cell wall and cell division of Bacillus cereus. Journal of Medical Microbiology 62(Pt7):1032-1037.
Santos OJ, Malafaia O, Ribas-Filho JM, Czeczko NG, Santos RH, Santos RA (2013b). Influence of *Schinus terebinthifolius* Raddi (Aroeira) and *Carapa guianensis* Aublet (andiroba) in the healing process of gastrorraphies. Arquivos Brasileiros de Cirurgia Digestiva 26(2):84-91. http://dx.doi.org/10.1590/S0102-67202013000200003
Santos FS (2009). As plantas Brasileiras, os jesuítas e os indígenas do Brasil: história e ciência na triaga brasílica (séc. XVII-XVIII). Casa do Novo Autor.
Santos SFA, Paulino VT, Katiki LM, Veríssimo CJ (2018). Perfil do produtor de leite da região de Joanópolis/SP: como ele lida com o controle do carrapato *Rhipicephalus microplus* e de outras doenças de importância veterinária. Pesquisa Veterinária Brasileira 38(1):77-88. http://dx.doi.org/10.1590/1678-5150-pvb-4825
Silva CES, Santos OJ, Ribas-Filho JM, Tabushi FI, Kume MH, Jukonis LB, Cella IF (2015). Effect of *Carapa guianensis* Aublet (Andiroba) and *Orbignya phalerata* (Babassu) in colonic healing in rats. Revista do Colégio Brasileiro de Cirurgiões 42(6):399-406. http://dx.doi.org/10.1590/0100-69912015006009
Silva HHG, Geris R, Rodrigues Filho E, Rocha C, Silva IG (2007). Larvicidal activity of oil-resin fractions from the Brazilian medicinal plant *Copaifera reticulata* Ducke (Leguminosae-Caesalpinoideae) against *Aedes aegypti* (Diptera, Culicidae). Revista da Sociedade Brasileira de Medicina Tropical 40(3):264-267. http://dx.doi.org/10.1590/S0037-86822007000300002
Silva IG, Zanon VOM, Silva HHG (2003). Larvicidal activity of *Copaifera reticulata* ducke oil-resin against *Culex quinquefasciatus* Say (Diptera: Culicidae). Neotropical Entomology 32(4):729-732. http://dx.doi.org/10.1590/S1519-566X2003000400029
Silva OS, Prophiro JS, Nogared JC, Kanis L, Emerick S, Blazius RD, Romão PR (2006). Larvicidal effect of andiroba oil, *Carapa guianensis* (Meliaceae), against *Aedes aegypti*. Journal of the American Mosquito Control Association 22(4):699-701.
Silva OS, Romão PR, Blazius RD, Prohiro JS (2004). The use of andiroba *Carapa guianensis* as larvicide against *Aedes albopictus*. Journal of the American Mosquito Control Association 20(4):456-457.
Silva WJM, Ferrari CKB (2011). Metabolismo Mitocondrial, Radicais Livres e Envelhecimento. Revista Brasileira de Geriatria e Gerontologia 14(3):441-451. http://dx.doi.org/10.1590/S1809-98232011000300
Smartt CT, Shin D, Kang S, Tabachnick WJ (2018). *Culex quinquefasciatus* (Diptera: Culicidae) From Florida Transmitted Zika Virus. Frontiers in Microbiology 9:e768. http://dx.doi.org/10.3389/fmicb.2018.00768
Smith RD, Sells DM, Stephenson EH, Ristic M, Huxoll DL (1976). Development of *Ehrlichia canis*, causative agent of canine ehrlichiosis, in the tick *Rhipicephalus sanguineus* and its differentiation from a symbiotic rickettsia. American Journal of Veterinary Research 37(2):119-126.
Soares AO, Souza AD, Feliciano EA, Rodrigues AF, D'agosto M, Daemon E (2006). Evaluation of ectoparasites and hemoparasites in dogs kept in apartments and houses with yards in the city of Juiz de Fora, Minas Gerais, Brazil. Revista Brasleira de Parasitologia Veterinária 15(1):13-16.
Spini VBMG, Ferreira FR, Paduani GF, Souza CS, Kerr WE (2006). Efeito da adição de castanha-do-pará à dieta de arroz e feijão sobre o ganho de peso em camundongos. Bioscience Journal 22(3):89-93.
Stachiw R, Ribeiro SB, Jardim MAG, Possimoser D, Alves WC, Cavalheiro WCS (2016). Potencial de produção de biodiesel com espécies oleaginosas nativas de Rondônia, Brasil. Acta Amazonica 46(1):81-90. http://dx.doi.org/10.1590/1809-4392201501151
Szabó MP, Cunha TM, Pinter A, Vicentini F (2001). Ticks (Acari: Ixodidae) associated with domestic dogs in Franca region, São Paulo, Brazil. Experimental and Applied Acarology 25(10):909-916. https://doi.org/10.1023/A:1020433003028
Teixeira FB, Brito Silva R, Lameira OA, Webber LP, D'almeida Couto RS, Martins MD, Lima RR (2017). Copaiba oil-resin (*Copaifera reticulata* Ducke) modulates the inflammation in a model of injury to rats' tongues. BMC Complementary and Alternative Medicine 17(1):e313. http://dx.doi.org/10.1186/s12906-017-1820-2
Tiosso PC, Carvalho AKF, Castro HF, Moraes FF, Zanin GM (2014). Utilization of immobilized lipases as catalysts in the transesterification of non-edible vegetable oils with ethanol. Brazilian Journal of Chemical Engineering 31(4):839-847. http://dx.doi.org/10.1590/0104-6632.20140314s00003006
Valdez IL, Farfan O, Sterner O, Turba AG (2009). Estudios preliminares de la caracterización química de ácidos grasos del aceite de frutos de *Bertholletia excelsa* por cromatografia de gases. Biofarbo 17(1):47-53.
Vendramini MC, Mathias MI, Faria AU, Furquim KC, Souza LP, Bechara GH (2012a). Action of andiroba oil (*Carapa guianensis*) on *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) semi-engorged females: morphophysiological evaluation of reproductive system. Microscopy Research and Technique 75(12):1745-1754. http://dx.doi.org/10.1002/jemt.22126
Vendramini MC, Camargo-Mathias MI, Faria AU, Bechara GH, Oliveira PR, Roma GC (2012b). Cytotoxic effects of andiroba oil (*Carapa guianensis*) in reproductive system of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) semi-engorged females. Parasitology Research 111(5):1885-1894. http://dx.doi.org/10.1007/s00436-012-3031-6
Vieira TMF, Regitano-D'arce MA. Antioxidant concentration effect on

stability of Brazil nut (*Bertholletia excelsa*) crude oil (1999). Archivos Latinoamericanos de Nutrición 49(3):271-274.
Villarreal JPV, Santos PR, Silva MAMP, Azambuja RHM, Gonçalves CL, Escareño JJH (2017). Evaluation of phytotherapy alternatives for controlling *Rhipicephalus (Boophilus) microplus in vitro*. Revista Brasileira de Parasitologia Veterinária 26(3):299-306. http://dx.doi.org/10.1590/s1984-29612017052
Wanzeler AMV, Júnior Sma GJT, Gouveia EHH, Henriques HYB, Chaves RH (2018). Therapeutic effect of andiroba oil (*Carapa guianensis* Aubl.) against oral mucositis: an experimental study in golden Syrian hamsters. Clinical Oral Investigations 22(5):2069-2079. http://dx.doi.org/10.1007/s00784-017-2300-2
Ziech RE, Farias LD, Balzan C, Ziech MF, Heinzmann BM, Lameira OA, Vargas AC (2013). Atividade antimicrobiana do oleorresina de copaíba (*Copaifera reticulata*) frente a *Staphylococcus coagulase* positiva isolados de casos de otite em cães. Pesquisa Veterinária Brasileira 33(7):909-913. http://dx.doi.org/10.1590/S0100-736X2013000700011.

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Evaluation of hepatoprotective, antioxidant and cytotoxic properties of aqueous extract of turmeric rhizome (Turmesac®)

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In the present study, the hepatoprotective, antioxidant and cytotoxic activities of aqueous extract of turmeric rhizome (Turmesac®) were evaluated. In brief, Turmesac® was extracted from turmeric rhizome. 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) assay was used to evaluate the hepatoprotective and cytotoxic activities of the Turmesac® against the human liver (HUH-7) and breast cancer (MCF-7) cell lines, respectively. The extract was further tested for antioxidant activity using the 2, 2-diphenyl-1-picrylhydrazy radical (DPPH) which was compared with standard butylated hydroxytoluene (BHT). Turmesac® showed significant cytotoxic potential against the MCF-7 cell lines with IC50 values of 163.19 µg/ml and show no cytotoxic potential against the HUH-7 cell lines. Turmesac® was used to stimulate hydrogen peroxide (H2O2-200 µM); a dose - dependent suppression of the toxic nature of H2O2 by the extract was observed. This confirmed the hepatoprotective potential of Turmesac®. The significant antioxidant activity of Turmesac® and standard BHT was observed at IC50 235.04 and 82.65 µg/mL, respectively. Further investigations will be carried out to evaluate the in vivo hepatoprotective activity of Turmesac®.

Key words: Turmesac®, antioxidant, hepatoprotective, cell viability.

INTRODUCTION

Turmesac (*Curcuma longa Linn*) is a member of the Zingiberaceae family and is cultivated in tropical and subtropical regions around the world. It is originates from India, Southeast Asia and Indonesia (Paramasivam et al., 2009). Turmeric is a plant that has a very long history of medicinal use, dating back nearly 4000 years. In Southeast Asia, turmeric is used not only as a principal spice but also as a component in religious ceremonies. Owing to its brilliant yellow color, turmeric is also known as Indian saffron. In the last 25 years, the importance of turmeric has been brought to lime light, as indicated by the over 3000 publications. According to the ayurvedic pharmacopoeia of India, essential oil from the rhizome of *curcuma longa* was used as a carminative, stomachic and tonic (The Ayurvedic Pharmacopoeia of India, 2001). Rhizomes are used as expectorant, cosmeceuticals,

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antiseptic, anthelmintic, blood purifier, and insecticide, spasmolytic and for the treatment of leprosy, hypertensive, cholera, syphilis, spleen disorders rheumatism, bronchitis, cough and cold (Kapoor, 1990). Recent studies have reported that curcumin effectively inhibits liver cirrhosis through its action on many pathways; for example, it inhibits the NF-κB pathway and reduces oxidative stress (Cai et al., 2017). Curcumin has antioxidant, anti-inflammatory, and anticarcinogenic pharmacological effects (Qiu et al., 2017). It acts by either interacting with molecular targets directly or altering gene expression and signaling pathways. Thus, curcumin has potential as a therapy for liver diseases. Curcumin, a polyphenolic pigment extracted from turmeric is a typical example because of its low toxicity and anticancer potency. The application of curcumin as a complementary therapy for ovarian cancer appears promising, because it induces apoptosis sensitivity to cisplatin in ovarian carcinoma without decreasing quality of life (Zhang et al., 2012; Sadzuka et al., 2012). Moreover, the enhancement of adaptive immunity was involved in curcuma-medicated tumor growth retardation (Luo et al., 2011; Bhattacharyya et al., 2010). In recent years, researches have confirmed that polysaccharides from natural products possess wide- ranging beneficial therapeutic effects and health-promoting properties. Recently, the polysaccharide is reported as a kind of effective free radical scavenger and antioxidant, playing a critical role in protecting against oxidation damage in living organisms. On the other hand, many diseases, such as asthma, chronic obstructive pulmonary disease, inflammation, diabetes, myocardial infarction, and cardiovascular diseases, are reported to be associated with oxidative stress (Scheibmeir et al., 2005; Olmez and Ozyurt, 2012; Sugamura and Keaney, 2011). In this study, aqueous extract of turmeric rhizome (Turmesac®) for hepatoprotective, antioxidant and cytotoxic activities were evaluated.

MATERIALS AND METHODS

Collection of plant material

Turmeric rhizome (Salem variety) were collected from Salem, November 2018, Tamilnadu, India and identified by K. Ramesh taxonomist, R &D division, Star Hi Herbs Pvt. Ltd, Jigani, Bangalore, Karnataka, India. The turmeric rhizome was dried at room temperature and powdered.

Preparation of the extract

The turmeric powder (250 g) was extracted with distilled water 100°C for 6 h. The extract was filtered, concentrated to remove the water under spray drying and the resulting powder was assayed for various biological activities.

Chemicals and reagents

Human hepatocellular adenocarcinoma (HUH-7) and human breast adenocarcinoma (MCF-7) cell line were purchased from NCCS, pune, India. Butylated hydroxyl toluene, 2,2-diphenyl-1-picrylhydrazy radical (DPPH), 5,5-dithiobis(2-nitrobenzoic acid), 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT), Reagent (Cat No: M6494, Invitrogen), DMSO (Cat No: D4540, Sigma), DMEM-High Glucose, Fetal Bovine Serum (FBS) and Antibiotic-Antimycotic (100x) were procured from Himedia, India. Analytical reagents and chemicals were purchased from Merck, India.

Cell lines and culture medium

The MCF-7 and HUH-7 stock cells were cultured and maintained using 89% DMEM-High glucose supplemented with 10% heat inactivated Fetal Bovine Serum (FBS) and 1% antibiotic-antimycotic (100x) and incubated at 37°C in a humidified atmosphere of 5% CO_2 until the cells reached 70 to 80% confluency. The cells were dissociated with cell 0.25% Trypsin-EDTA.

MTT cytotoxicity assay

The MTT assay determined by the reduction of yellow 3-(4, 5-dimethythiazol2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product (Alley et al., 1986). The cells are then solubilised with an organic solvent (*e.g.* isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. The viability of the cells was checked using Hemacytometer prior to MTT Assay. 200 µL of cell suspension was seeded in a 96-well plate at the required cell density (20,000 cells per well in media), without the test agent and allowed to grow for about 24 h.

After 24 h, Turmesac® extract (25, 50, 100, 200 and 400 µg/mL) in media was added to treat the cells and incubated for another 24 h at 37°C in 5% CO_2 atmosphere. Camptothecin with a concentration of 25 µM was used as a positive control. After the incubation period, the morphology of the cells was observed using inverted biological microscope and images of the cells was captured at 10x magnification. Thereafter, the spent media was removed and 100 uL of MTT reagent (0.5 mg/mL) was added to each well and incubated for 3 h at 37°C. Thereafter, the formed formazone crystals were dissolved with 100 µl of DMSO and the absorbance readings were taken by ELISA Reader (ELX 800, Biotek) at 570 nm.

The viability of the cells was determined by the following formula:

% of viability = (Mean OD of test compound treated Cells/Mean OD of untreated cells) x 100

Hepatoprotective activity

Briefly, 200 µL of cell suspension was seeded in a 96-well plate at required cell density (20,000 cells per well), without the test agent and the cells were allowed to grow for about 24 h. 200 uM of H_2O_2 was added to all the wells except the untreated wells and incubate for 4 h to stimulate stress to cells and appropriate concentrations of test compound (25, 50, 100, 200 and 400 µg/mL) was added and incubated for 24 h at 37°C in a 5% CO_2 atmosphere. After the incubation period, 100 µL of MTT reagent (5 mg/mL) was added and incubated for 3 h at 37°C. After incubation period, the formed formazan crystals were dissolved with 100 µL of DMSO and the absorbance readings were taken by a spectrophotometer or an

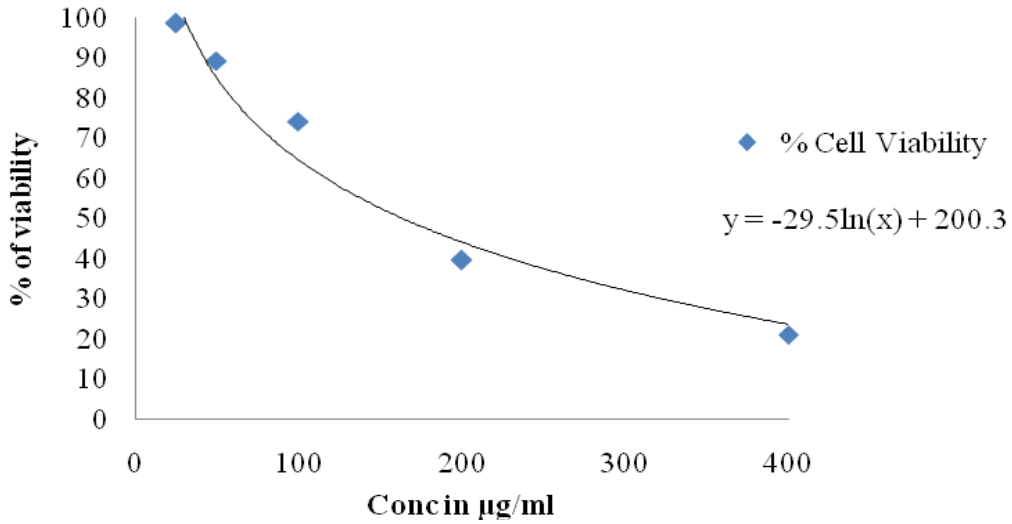

Figure 1. Scatter graph showing the % of cell viability of MCF7 cell line against the Turmesac® and the inhibitory concentration (IC_{50} value) observed is163.19 µg/ml.

ELISA reader at 570 nm (Torres- González et al., 2016). The viability of the cells was determined by the following formula:

% of viability = (OD of test compound/OD of untreated cells) × 100

Determination of antioxidant activity

The free radical scavenging activity of Turmesac® was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method of Blois (1958). A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol at different concentrations (50, 100, 150, 200 and 250µg/mL). After 30 min, absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as the reference material. Percentage DPPH radical scavenging activity was calculated by the following equation. The radical scavenging activity of DPPH was calculated using the following equation:

% of DPPH radical scavenging activity = $[(A_0 - A_1)/A_0] \times 100$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard. Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated.

Ultraviolet detection of turmeric polysaccharides

Preparation of blank solution

1 ml of distilled water was added to 1 ml of 5% phenol followed by 5 ml of concentrated H_2SO_4.

Preparation of standard solution

A stock solution of glucose 100 µg/ml was prepared in distilled water. Aliquots were taken from this solution to obtain sugar concentrations of 60 to 90 µg/ml. 1 ml of test solution was added and 1 ml of 5% phenol solution followed by 5 ml of concentrated H_2SO_4. The absorbance was measured after 10 min at 488 nm against blank (Sharma et al., 2012).

Preparation of test sample

10 mg of sample dissolved in 100 ml of distilled water.1 ml of phenol solution was added; 1 ml of test solution was added, followed by 5 ml of concentrated H_2SO_4. The absorbance was measured after 10 minutes at 488 nm against blank.

RESULTS AND DISCUSSION

Cytotoxic activity

The results of the cytotoxicity screened by MTT assay against MCF-7 and HUH7 cell lines of the Turmesac® is given in Figures 1 and 2. The formulated extract exhibited significant cytotoxicity against the MCF-7 cell line with IC_{50} value of 163.19 µg/mL and does not show any cytotoxicity against the HUH-7 cell lines within the incubation period of 24 h in the concentrations ranging from 25 to 400 µg/mL, respectively. These findings suggest that the reduction observed in the viable cells following treatment with Turmesac® is due to cell death against the MCF-7 cell line. The leading cause of cancer is breast cancer, which holds fifth place in cancer-related deaths globally (Fitzmaurice et al., 2017). The high cost of chemotherapy for treating breast cancer and its side effects make this disease one of the most challenging (Blumen et al., 2016). The present observations provide preliminary data revealing that Turmesac® has potent cytotoxic activity against MCF-7 cells. The study serves as a scientific data for the use of Turmesac® chemotherapeutic properties as well as promising anticancer drugs.

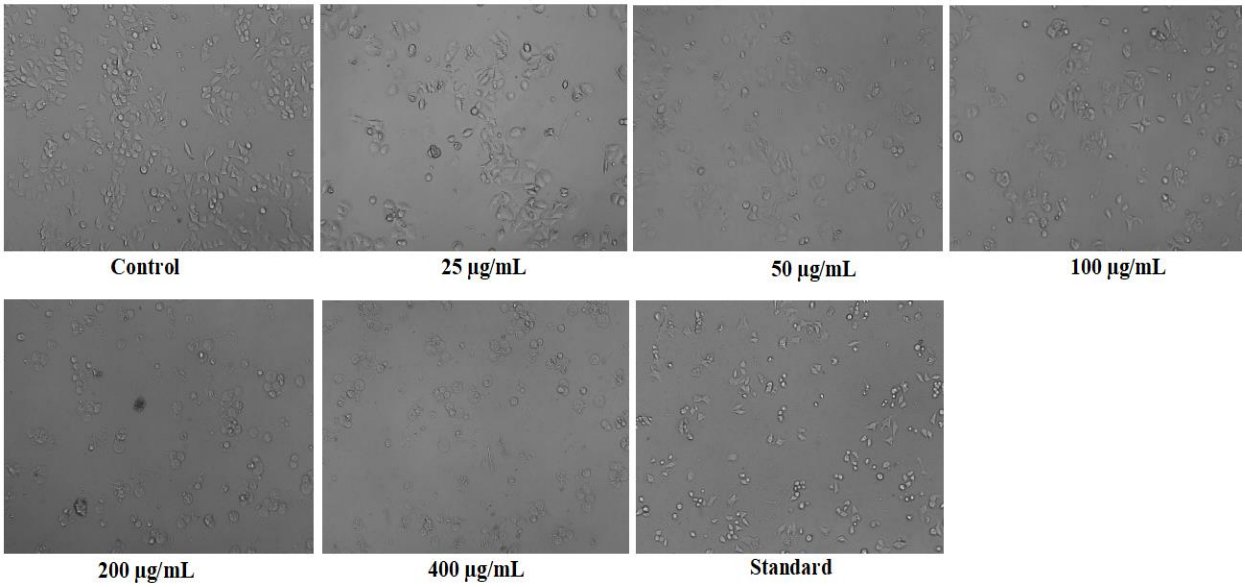

Figure 2. Direct microscopic observations of MCF-7 cells treated with Turmesac® visualized under inverted biological microscope with 10x magnification at the concentrations ranging from 25 to 400 µg/ml compared to Untreated control and standard Camptothecin.

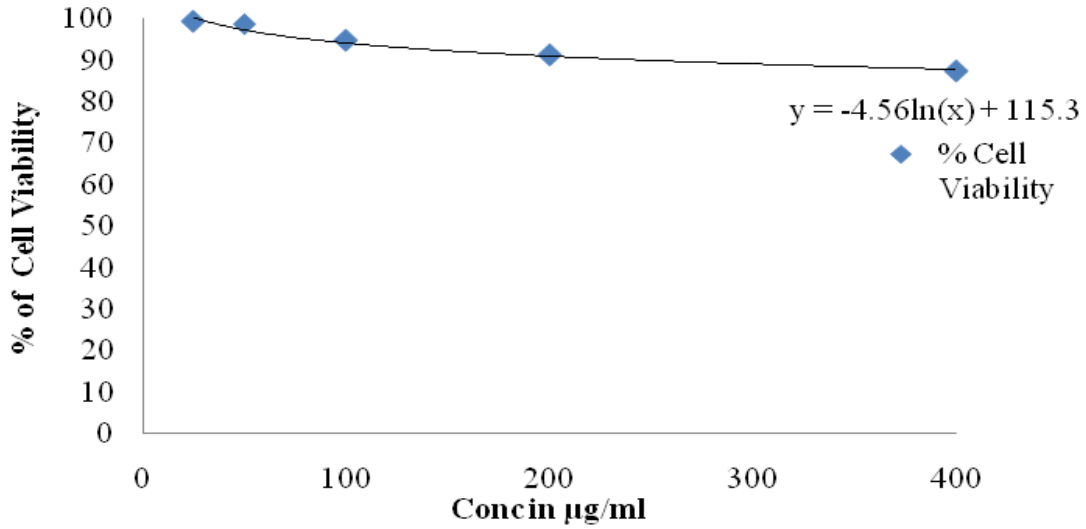

Figure 3. Cytotoxicity effect of Turmesac against HUH7cell line after 48 h incubation.

Hepatoprotective activity

Hepatoprotective activity of the Turmesac® on HUH-7 cells from Human Liver cells was investigated *in vitro* 3-(4) 5-Dimethyl-thiazol-Zyl) - 2,5 biphenyl tetrazolium bromide (MTT) assay (Figures 3 and 4). In MTT assay, 25 to 400 µg/mL different concentrations were used by stimulating the cells with 200 µM of H_2O_2 for 24 h. The hepatoprotective activity of Turmesac® was evaluated using well-maintained HuH7 cells. H_2O_2 was used as hepatotoxicant and silymarin was used as a standard positive control. The hepatoprotective activity of silymarin and Turmesac® were found to be IC_{50} value 8.7 and 150.8 µg/mL, respectively. Shapiro et al. (2006) reported that curcumin showed the hepatoprotective effect and decreased levels of thiobarbituric acid reactive substances (TBARS), minimized oxidative stress and inhibited inducible nitric oxide (iNOS) protein and NF-κB in acute thioacetamide hepatotoxicity rats supplemented with 200 and 400 mg/kg per day curcumin. Curcumin

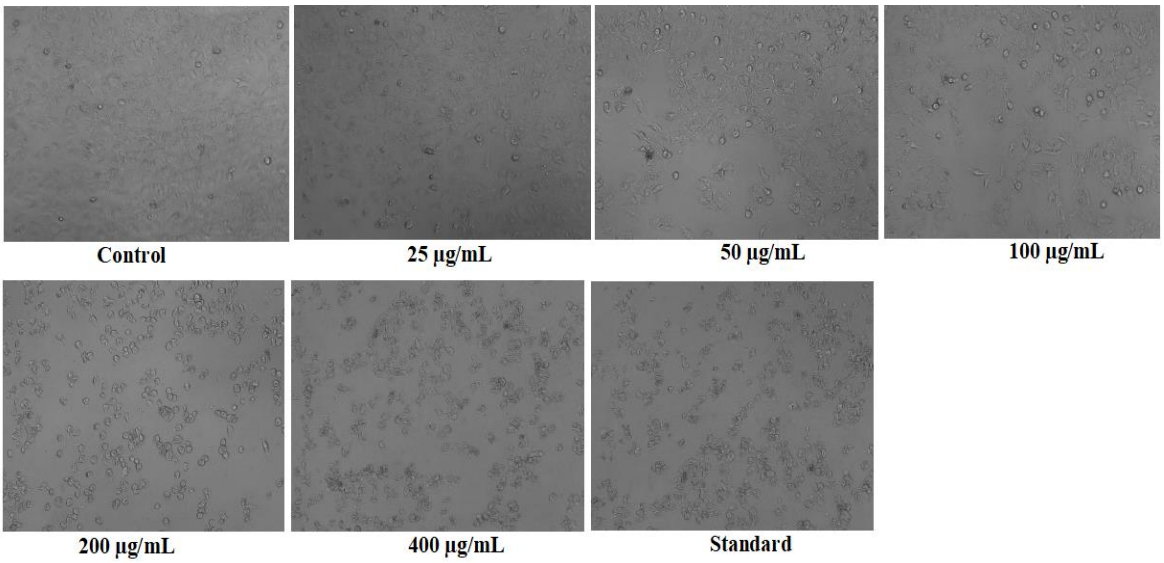

Figure 4. Direct microscopic observations of HUH7 cells treated with Turmesac visualized under inverted biological microscope with 10x magnifications at the concentrations ranging from 25 µg to 400 µg/ml compared to untreated control and standard Silymarin.

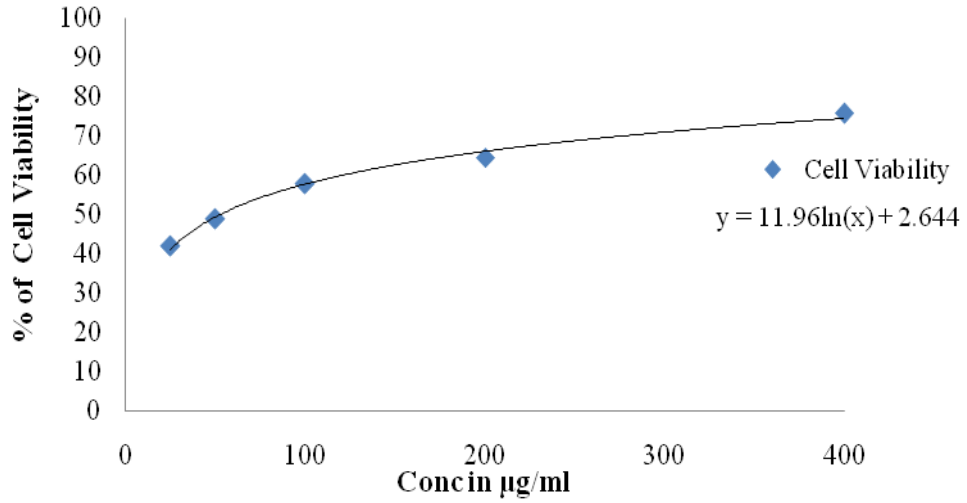

Figure 5. Cytotoxicity effect of Turmesac® vs H_2O_2 treated HUH7cell line after 48 h incubation.

treatment shows hepatoprotective activity induced by *E. histolytica*, decreasing serum activities of ALT, ALP and *γ*-GTP, which were consistent with macroscopic and microscopic observations, suggesting that curcumin protects in both the early and late stages of liver infection (Deng et al., 2016). Several reports suggest that, the curcumin protects the liver against hepatotoxic compounds, such as carbon tetrachloride, alcohol and paracetamol (RVaratharajalu et al., 2016; Fernandez-Rojas et al., 2016). Turmeric is used as a traditional medicine and it has a therapeutic potential against jaundice and other disorders like liver, parasitic infections, ulcers, various skin diseases etc. The rhizome juice from *C. longa* is very useful in the treatment of many diseases such as anthelmintic, asthma, gonorrhea and urinary infections. Its essential oil is also used in the treatment of carminative, stomachic and tonic (Phansawan and Poungbangpho, 2007; Salama et al., 2013). The Turmesac® showed significant hepatoprotective activity against the HUH-7 cell lines by suppressing the toxic potential of H_2O_2 with increased concentrations of extract and ensuring the significant protective against the Human Liver Cell line (Figures 5 and 6).

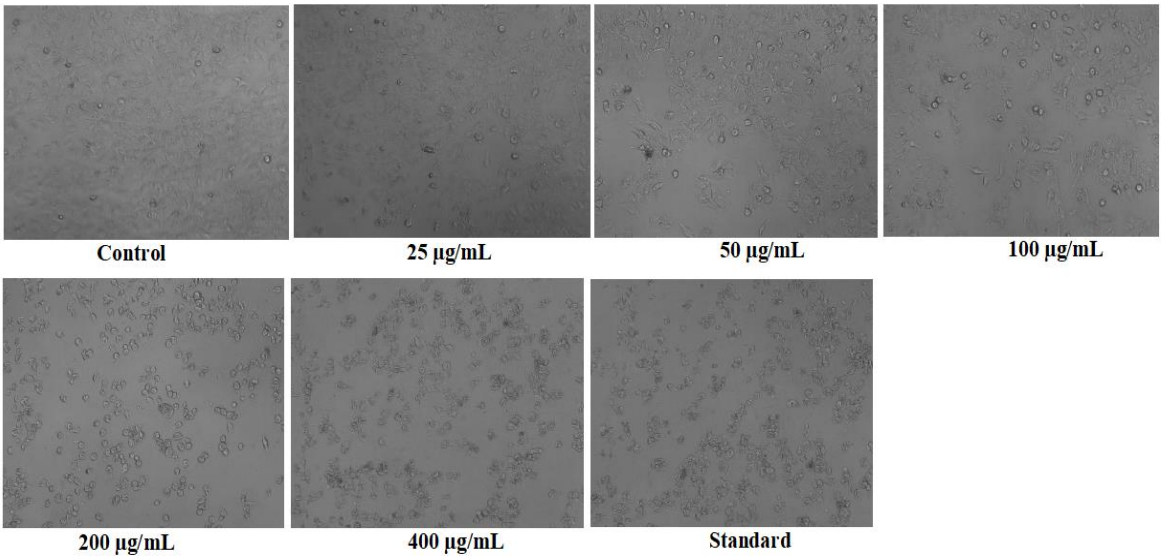

Figure 6. Direct microscopic observations of H_2O_2 stressed HUH7 cells treated with Turmesac®visualized under Inverted Biological microscope with 10x Magnification at the concentrations ranging from 25 µg to 400 µg/ml compared to untreated control and standard Silymarin.

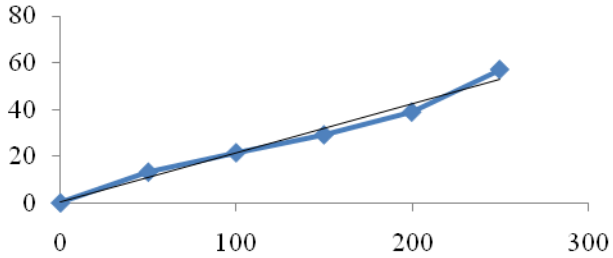

Figure 7. DPPH free radical scavenging activity (IC_{50}µg/ml) of Turmesac®.

Table 1. DPPH free radical scavenging activity (IC_{50}µg/ml) of Turmesac® and standard BHT.

S/N	BHT (µg/ml)	OD (at 517 nm)	% inhibition of BHT	Sample concentration (µg/ml)	OD (at 517 nm)	% of inhibition of Turmesac®
1	Control	0.522	-	Control	0.493	0
2	20	0.464	11.11	50	0.428	13.18
3	40	0.367	29.69	100	0.387	21.5
4	60	0.313	40.03	150	0.349	29.2
5	80	0.273	47.7	200	0.301	38.94
6	100	0.222	57.47	250	0.211	57.2
		IC_{50} 82.65 µg/ml		**IC_{50} 235.04 .µg/ml**		

Antioxidant activity

The antioxidant activity of the Turmesac® is shown in Figure 7 and Table 1. The extract Turmesac® antioxidant activity was compared with standard butylated hydroxytoluene (BHT). The free radical antioxidant scavenging activity of standard BHT and Turmesac® were found to be IC_{50} values of 82.65 and 235.04 µg/ml,

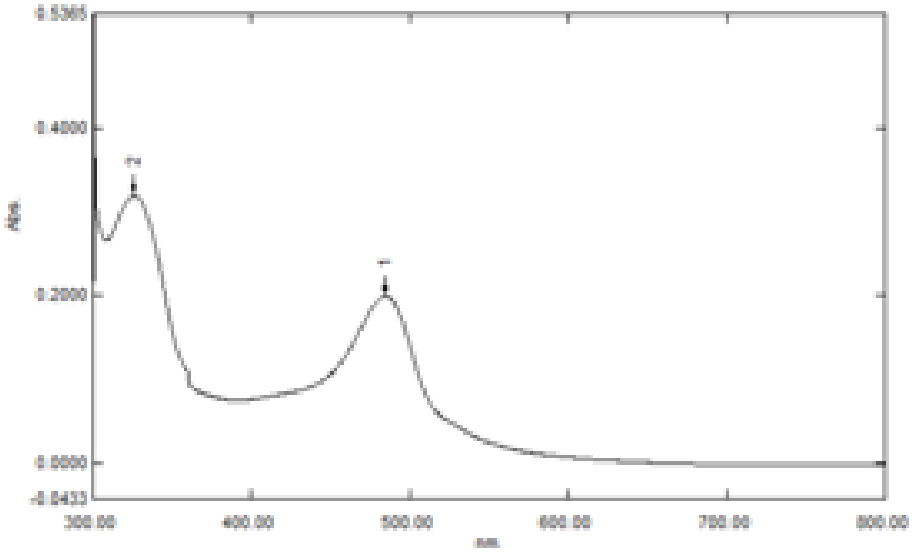

Figure 8. UV chromatogram of turmeric polysaccharides.

respectively. Antioxidants are tremendously important substances which possess the ability to protect the body from damage causing free radical induced oxidative stress. Free radicals are associated with many diseases. Hydroxyl, DPPH and superoxide radical scavenging activities are widely used to the quantitative determination of antioxidant capacities of biological samples and foods (Rahman and Islam 2013). The DPPH method was first introduced by Blois in 1958 and this method is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity. Most of the recent published papers are focused on the strategies to enhance the curcumin bioavailability. Its potent antioxidant properties are because of its chemical structure which consists of two methoxylated phenols that are connected by α, β unsaturated carbonyl groups (Priyadarsini, 2014). It has also been reported that the scavenging oxygen radical by GSH is related to the repair and neutralization of ROS induced damage (Nimse and Pal, 2015). One of these strategies is the preparation of curcumin complexes with natural compounds including piperine, or quercetin. For this reason, the study tried to prepare the turmeric polysaccharides to improve the antioxidant properties. The Turmesac® were screened for the presence of bioactive compound UV chromatogram showed the presence of polysaccharides (Figure 8).

Conclusion

The present investigation indicates that Turmesac® shows promising properties for antioxidant, cytotoxicity and hepatoprotective activities against MCF-7 and HUH-7 cells, respectively. The antioxidant, cytotoxic and hepatoprotective role of Turmesac® is due to its polysaccharides. The study serves as a scientific data for the use of Turmesac® exhibited significant activity of antioxidant, cytotoxic and hepatoprotective drug.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Alley MC, Scudiere DA, Monks A, Czerwinski M, Shoemaker R, Boyd MR (1986). Validation of an automated microculture tetrazolium assay (MTA) to assess growth and drug sensitivity of human tumor cell lines. Proceedings of the American Association for Cancer Research 27:389-391.

Bhattacharyya S, Hossain DMS, Mohanty S, Sen GS, Chattopadhyay S, Banerjee S, Chakraborty J, Das K, Sarkar D, Das T, Sa G (2010). Curcumin reverses T cell- mediated adaptive immune dysfunctions in tumor-bearing hosts. Cellular Aand Molecular Immunology 7(4):306-315.

Blois MS (1958). Antioxidant determinations by the use of a stable free radical. Nature 29:1199-1200.

Blumen H, Fitch K, Polkus V (2016). Comparison of treatment costs for breast cancer by tumor stage and type of service. American Health and Drug Benefits 9(1):23-32.

Cai Y, Di L, Yanting Z, Chaohui Z, Hongchun L, Chuantao T, Feng L, Lili L, Shuncai Z (2017). Curcumin protects against intestinal origin endotoxemia in rat liver cirrhosis by targeting PCSK9. Journal of Food Science 82(3):772-780.

Deng B, Sullivan MA, Chen C et al. (2016). Molecular structure of human-liver glycogen. PLOS ONE 11(3):e0150540.

Fitzmaurice C, Allen C, Barber RM , Barregard L, Bhutta ZA, Brenner H, Dicker DJ (1990 to 2015). A systematic analysis for the global burden of Disease Study. JAMA Oncology 3(4):524-548.

Government of India Ministry of Health and Family Welfare (2001). The Ayurvedic Pharmacopoeia of India: the Controller of Publications Civil Lines New Delhi, Immunology 27:19-35.

Kapoor LD (1990). Hand book of ayurvedic medicinal plants. Boca

Raton (FL.), CRC Press.
Luo F, Song X, Zhang Y, Chu Y (2011). Low-dose curcumin leads to the inhibition of tumor growth via enhancing CTL-mediated antitumor immunity. International Immunopharmacol 11: 1234-1240.
Nimse SB, Pal D (2015). Free radicals, natural antioxidants, and their reaction mechanisms. Royal Society of Chemistry Advances 5(35):27986-28006.
Olmez I, Ozyurt (2012). Reactive oxygen species and ischemic cerebrovascular disease. Neurochemistry International 60(2):208-212.
Paramasivam M, Poi R, Banerjee H, Bandyopadhyay A (2009). High performance thin layer chromatographic method for quantitative determination of curcuminoids in Curcuma longa germplasm. Food Chemistry 113: 640-644.
Phansawan B, Poungbangpho S (2007). Antioxidant capacities of Puerariamirifica, Steviarebaudiana Bertoni, Curcuma longa Linn. *Andrographis paniculata* (Burm. f.) Nees and Cassiaalata Linn. for the development of dietary supplement. Kasetsart Journal 41(3):407-413.
Priyadarsini KI (2014). The chemistry of curcumin: from extraction to therapeutic agent. Molecules 19(12):20091-20112.
Qiu P, Sun J, Man S (2017). Curcumin attenuates N-nitrosodiethylamine-induced liver injury in mice by utilizing the method of metabonomics. Journal of Agricultural and Food Chemistry 65(9):2000-2007.
Rahman MA, Islam MS (2013). Antioxidant, antibacterial and cytotoxic effects of the phytochemical of whole *Leucas aspera* extract. Asian Pacific Journal of Tropical Biomedicine 3(4):273-279.
Salama SM, Abdulla MA, AlRashdi SA, Ismail S, Alkiyumi (2013). Hepatoprotective effect of ethanolic extract of *Curcuma longa* on thioacetamide induced liver cirrhosis in rats. BMC Complementary and Alternative Medicine 5(13):56.
Scheibmeir HD, Christensen SH, Whitaker J, Jegaethesan R, Clancy JD, Pierce (2005). A review of free radicals and antioxidants for critical care nurses. Intensive and Critical Care Nursing 21(1):24-28.
Sharma K, Agrawal SS, Gupta M (2012). Development and Validation of UV spectrophotometric method for the estimation of Curcumin in Bulk Drug and Pharmaceutical Dosage Forms. International and Research 4(2):375-380.
Shapiro H, Ashkenazi M, Weizman N, Shahmurov M, Aeed H, Bruck R (2006). Curcumin ameliorates acute thioacetamide-induced hepatotoxicity. J. Gastroenterol. Hepatology 21(2):358-366.
Sugamura JF, Keaney JR (2011). Reactive oxygen species in cardiovascular disease Free Radical. Biology and Medicine 51(5):978-992.
Torres-González L, Muñoz-Espinosa LE, Rivas-Estilla AM, Trujillo-Murillo K, Salazar-Aranda R, Waksman N, Torres D, Cordero-Pérez P (2016). Protective effect of four Mexican plants against CCl 4-induced damage on the Huh7 human hepatoma cell line. Annals of Hepatology 10:73-79.
Varatharajalu R, Garige M, Leckey LC, Reyes-Gordillo K, Shah R, Lakshman MR (2016). Protective role of dietary curcumin in the prevention of the oxidative stress induced by chronic alcohol with respect to hepatic injury and antiatherogenic markers. Oxidative Medicine and Cellular Longevity 2016:ID 5017460.
Zhang X, Zhang HQ, Zhu GH et al. (2012). A novel mono-carbonyl analogue of curcumin induces apoptosis in ovarian carcinoma cells via endoplasmic reticulum stress and reactive oxygen species production. Molecular Medicine Reports 5(3):739-744.

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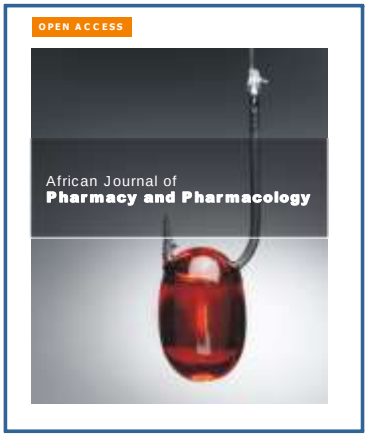

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