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Review

Compounds isolated from *Harpalyce brasiliiana* Benth and their pharmacological properties

Carla Brugin Marek^{1*} and Ana Maria Itinose²

¹Laboratory of Cellular Toxicology, Western Paraná State University, 85819110, Cascavel, Brazil.

²Assistance Center in Toxicology (CEATOX), Western Paraná University Hospital, 85806470, Cascavel, Brazil.

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***Harpalyce brasiliiana* Benth, known as snakeroot, is one of the most popular herbal medicines against snakebite in South America. A hydroalcoholic solution is traditionally prepared from the roots of *H. brasiliiana*. In the last two decades, understanding the pharmacological properties and the possible medicinal applications of *H. brasiliiana* has increased considerably. *H. brasiliiana* has antivenom activity and anticarcinogenic, antimicrobial and antioxidant properties. Various *in vitro* and *in vivo* studies have shown *H. brasiliiana*'s diverse biological properties and its potential for disease treatment. The different biological effects of this plant may be attributable to the presence of secondary active metabolites such as pterocarpan, triterpenoids, chalcones and flavonoids. This overview presents different aspects of this plant and the pharmacological properties of its compounds through a review of the available literature. The results support the use of *H. brasiliiana* in the treatment of snakebite and its potential for treatment of other diseases in folk medicine.**

Key words: Snake antivenom, medicinal plants, pterocarpan.

INTRODUCTION

Harpalyce brasiliiana Benth (family Leguminosae), commonly known as snakeroot, is a shrub used in South America, particularly in northeastern Brazil to treat snake bite poisoning. The local people have reported that hydroalcoholic solution from the roots of *H. brasiliiana* is an antidote against snakebite (Silva et al., 1999). Much of the activity of *H. brasiliiana* is because of pterocarpan, which are major compounds present in the plant. Pterocarpan play an important role as phytoalexins and have significant pharmacological applications. Pterocarpan are present in the roots and leaves (Jiménez-González et al., 2008).

Initial studies using the roots of *H. brasiliiana* showed

two active compounds, betulinic acid and a prenylated pterocarpan (Silva et al., 1997). Subsequently, other bioactive pterocarpan derivatives were isolated, including 4'-dehydroxycabenegrin A-I, cabenegrin A-I, cabenegrin A-II, leiocarpin, medicarpin and maackiain (Silva et al., 2004; Militão et al., 2007). The active constituents isolated from this plant have pharmacological activities such as antiviral, antiophidic and anticarcinogenic activities. Two polyphenolic compounds, harpalycin and quercetin, were isolated from the *H. brasiliiana* leaves (Silva et al., 1999). These compounds have numerous biological activities in animal cells, such as free radical scavenging properties and antioxidant and anticholinesterase activities

*Corresponding author. E-mail: carla.marek@unioeste.br. Fax: +55 45 33215407.

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Table 1. Compounds isolated from *Harpalyce brasiliiana* Benth.

Extract type	Plant part	Compound	Reference
	Roots	4'-dehydroxycabeneigrin A-I	Silva et al. (1997)
		3-hydroxy-4-isopentenyl-8,9-methylenedioxypterocarpan	Silva et al. (1999)
		3 β -hydroxy-20(29)-lupen-28-oic acid or betulinic acid	Silva et al. (1997; 1999)
	Leaves	Harpalycin and quercetin 5,7,3',4'-tetrahydroxyflavonol	Silva et al. (1999)
Ethanollic	Roots	Leiocarpin, medicarpin, maackiain, cabeneigrin A-I and cabeneigrin A-II	Militão et al. (2007)
		(-)-2-geranyl-3-hydroxy-8,9-methylenedioxypterocarpan	Vieira et al. (2008)
	Roots	(-)-6aR,11aR-2(4',5'-dihydroxy-1'-isopentenyl)-3-hydroxy-8,9-methylenedioxypterocarpan and (-)-6aR,11aR-2(4'-hydroxy-1'-isopentenyl)-3-hydroxy-8,9-methylenedioxypterocarpan	Araújo et al. (2008)
		2-Geranyl-2,3',4,4'-tetrahydroxychalcone	Vieira et al. (2008)
		(-)-7,8,3',4'-trihydroxy-8-(3",7"-dimethyl-octa-2",6"-dienoyl)-flavanone	Araújo et al. (2008)

activities (Djeridane et al., 2006; Katalanić et al., 2010; Ximenes et al., 2012). Although few studies have been performed on this plant to date, the results show that *H. brasiliiana* is a source of several bioactive compounds of medicinal interest. Here, an overview of the available literature on the main properties of this plant and the compounds isolated from this plant has been presented.

ISOLATED COMPOUNDS

Studies have been performed, particularly during the past two decades, to isolate and identify bioactive compounds from *H. brasiliiana* (Table 1). Phytochemical studies using leaves and roots of *H. brasiliiana* showed the presence of several substances belonging to the class of flavonoids, particularly pterocarpan and some triterpenoids. Ethanollic extract from roots showed a prenylated pterocarpan, namely 4'-dehydroxycabeneigrin A-I (Silva et al., 1997). The structure of this pterocarpan

is similar to that of the known snake venom antidote, cabeneigrin A-I (Figure 1), a compound initially isolated by Nakagawa et al. (1982) from a hydroethanollic beverage called "Específico Pessôa," a Brazilian folk medicine used against snakebite. Subsequently, other pterocarpan were also isolated from the ethanollic extract roots. They are 3-hydroxy-4-isopentenyl-8,9-methylenedioxypterocarpan (Silva et al., 1999); leiocarpin, medicarpin, maackiain, cabeneigrin A-, and cabeneigrin A-II (Militão et al., 2007); (-)-2-geranyl-3-hydroxy-8,9-methylenedioxypterocarpan (Vieira et al., 2008); (-)-6aR,11aR-2(4',5'-dihydroxy-1'-isopentenyl)-3-hydroxy-8,9-methylenedioxypterocarpan and (-)-6aR,11aR-2(4'-hydroxy-1'-isopentenyl)-3-hydroxy-8,9-methylenedioxypterocarpan (Araújo et al., 2008). The compounds isolated from the roots included the triterpenoid 3 β -hydroxy-20(29)-lupen-28-oic acid or betulinic acid (Silva et al., 1997; Silva et al., 1999); the chalcone 2-geranyl-2,3',4,4'-tetrahydroxychalcone (Vieira et al., 2008)

and the flavanone (-)-7,8,3',4'-trihydroxy-8-(3",7"-dimethyl-octa-2",6"-dienoyl)-flavanone (Araújo et al., 2008). The isoflavone harpalycin and the flavonol quercetin 5,7,3',4'-tetrahydroxyflavonol were isolated from the leaves of *H. brasiliiana* (Silva et al., 1999).

PTEROCARPANS

Pterocarpan are phytoalexins, that is substances produced *de novo* by plants to provide resistance against stress factors such as microbial infections or stress of abiotic origin. The second largest class of natural isoflavonoids found in the Leguminosae family is characterized by the presence of a tetracyclic system of benzofuran-benzopyran rings with two chiral centers in the 6a and 11a positions in *H. brasiliiana* (Jiménez-González et al., 2008). All natural pterocarpan have a junction of *cis*-fused rings. Computational studies have shown that the *trans* isomers are

Table 2. Compounds and their pharmacological activities

Compound	Pharmacological Activity	Reference
Pterocarpan		
Cabenegrin A-I and A-II	Anti-inflammatory, antifungal, antimicrobial, antiparasitic, antimitotic, anticarcinogenic, and antiophidic against <i>Bothrops atrox</i>	Nakagawa et al. (1982), Jiménez-González et al. (2008), Zhou et al. (2009), Harinantenaina et al. (2010) and Dey and De (2012)
4'-dehydroxycabenegrin A-I Leiocarpin C	Antimytotoxic, antiproteolytic, and PLA2 inhibitor against <i>B. jararacussu</i> (<i>in vitro</i>) Cytotoxic activity against several tumor cell lines	Ishiguro et al. (1982) and Silva et al. (2004) Yadav et al. (2008)
Maackiain	Inhibitor of aryl hydrocarbon hydroxylase Inhibitory action on lymphoblastoid cell lines I Induction of apoptosis of the DNA of human promyelocytic leukemia HL-60 cells	Gelboin et al. (1981) Skinnider and Stoessl (1986) Aratanechemuge et al. (2004)
Medicarpin	Inhibitor of mixed function oxidase (P450) (<i>in vitro</i>) Suppressor of osteoclastogenesis Stimulator of osteoblast differentiation	Friedman et al. (1985) Tyagi et al. (2010) Bhargavan et al. (2012)
Triterpenoid		
Betulinic acid	Anticarcinogenic	Chintharlapalli et al. (2007)
Chalcones	Anti-inflammatory, antimicrobial, antioxidant, and anticarcinogenic	Hijova (2006) and Perjési and Rozmer (2011)
Chalcone 2-hydroxychalcone	Anticarcinogenic Preventive effect on diabetic complications	Makita et al. (1996) Jamal et al. (2009)
Flavonoids		
Quercetin	Antioxidant Inhibitor of butyrylcholinesterase	Day et al. (2000) Katalinić et al. (2010)
4'-chloroflavanone	Antiproliferative activity	Choi et al. (2010)
Naringenin	Antioxidant (<i>in vitro</i>)	Cavia-Saiz et al. (2010)
Naringenin and Hesperidin	Inhibition of bone resorption	Habauzit et al. (2011)
Naringenin flavanone	Antihyperglycemic and activity on bone metabolism	Annadurai et al. (2012)
Harpalycin 2 isoflavone	Anti-inflammatory	Ximenes et al. (2012)

energetically unfavorable, which explains the reason for all pterocarpan of natural origin possessing the 6a, 11a-*cis* configuration (Van Aardt et al., 2001).

The main bioactivities of pterocarpan (Table 2) included anti-inflammatory, antifungal, antimicrobial,

antiparasitic, antimitotic, anticarcinogenic and antiophidic activities (Nakagawa et al., 1982; Jiménez-González et al., 2008; Zhou et al., 2009; Harinantenaina et al., 2010; Dey and De, 2012).

Cabenegrins A-I and A-II were isolated initially by Nakagawa et al. (1982) from "Específico

Pessôa," a northeast Brazilian folk medicine used against snakebite and spider venom. According to the authors, it was a hydroethanolic extract from the root of the plant popularly known as "black head". Cabenegrin A-I and cabenegrin A-II showed antiophidic activity at a dose of 2.0

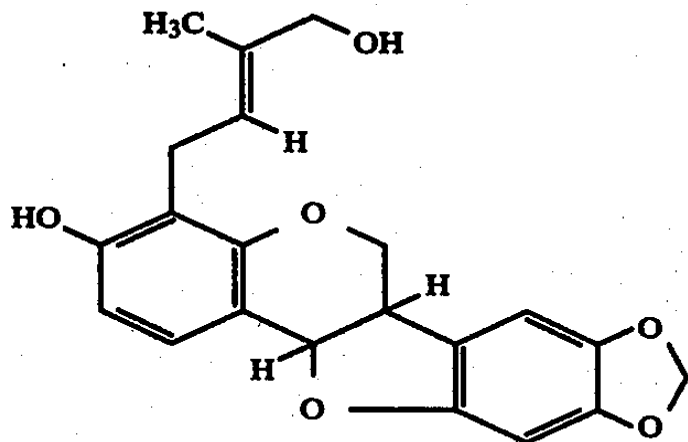


Figure 1. Cabenegrin A-I, a compound initially isolated by Nakagawa et al. (1982) and Darko (1984).

to 2.8 mg/kg in mice and at a dose of 1.0 mg/kg in beagle dogs previously envenomed using the venom of *Bothrops atrox* (Viperidae). Further, on the basis of spectroscopic analysis of cabenegrins, other pterocarpanes were obtained by synthesis. The compound 4'-dehydroxycabenegrin A-I obtained by synthesis showed antimyotoxic, antiproteolytic and PLA2 inhibitor activities against *Bothrops jararacussu* (Viperidae) crude venom *in vitro* (Ishiguro et al., 1982; Silva et al., 2004).

The pterocarpan medicarpin is naturally found in dietary legumes and has also been synthesized for pharmacological studies. Studies on rat liver microsomes showed that medicarpin can inhibit mixed-function oxidase (P450) activity, particularly the cytochrome P-450-dependent aryl hydrocarbon hydroxylase (AHH) and ethoxycoumarin deethylase (ECD) activities (Friedman et al., 1985). Recent studies have shown that medicarpin affects osteoclastogenesis. Tyagi et al. (2010) showed that medicarpin at a dose of 10^{-10} mol/L suppresses osteoclastogenesis in bone marrow cells and induces apoptosis of mature osteoclasts in ovariectomized mice. The mechanism of action appeared to be independent of estrogen receptor activation. Medicarpin acted as an estrogen receptor agonist; however it showed no uterine estrogenicity. Medicarpin stimulates osteoblast differentiation and promotes achievement of peak bone mass in rats (Bhargavan et al., 2012).

Various studies have shown that maackiain inhibits different forms of aryl hydrocarbon hydroxylase in human and rat livers (Gelboin et al., 1981), besides its inhibitory effect on the growth of human lymphoblastoid cell line I (Skinnider and Stoessl, 1986) and induction of apoptosis in the DNA of human promyelocytic leukemia HL-60 cells (Aratanechemuge et al., 2004). Leiocarpin C, isolated from the stem bark of the *Goniothalamus leiocarpus* (Annonaceae), also possesses cytotoxic activity against several human tumor cell lines (Yadav et al., 2008). Studies

on three human cells lines, namely HL-60 (leukemia cells), HCT-8 (colon cancer cells) and MDA-MB-435 (melanoma cells) showed that leiocarpin is cytotoxic and its half-maximal inhibitory concentration (IC_{50}) values are 5.5, 6.9 and 13.7 $\mu\text{g/ml}$, respectively (Militão et al., 2007). Studies on understanding the synthesis of this compound have been reported (Yadav et al., 2008; Krishna and Alivelu, 2011).

TRITERPENOIDS

Triterpenoids are natural products, with thirty carbons, derived from a squalene skeleton or enzymatic reaction by cyclization products of squalene, oxidosqualene and bis-oxidosqualene. They are found either in the acyclic or cyclic forms and tetracyclic and pentacyclic triterpenoids the most common forms of triterpenoids (Xu et al., 2004). Triterpenoids from natural origin and their synthetic derivatives are pharmacologically active (Table 2) and have anti-inflammatory and anticarcinogenic activities (Bishayee et al., 2011). The triterpenoid 3β -hydroxy-20(29)-lupen-28-oic acid, betulinic acid inhibits the growth of multiple tumors, including human melanomas. In an experimental model of prostate cancer, betulinic acid treatment decreased the growth of the cancer cells and its IC_{50} value was 1 to 5 $\mu\text{mol/L}$ (Chintharlapalli et al., 2007). In addition, the anticarcinogenic effect of betulinic acid on colon cancer cells has also been investigated. The results showed that the betulinic acid at a concentration of ≥ 5 $\mu\text{mol/L}$ inhibited the growth of cancer cells and down-regulated the specificity protein (Sp) transcription factors (Chintharlapalli et al., 2011).

CHALCONES

Chalcones, 1,3-diphenyl-2-propenones, considered as precursors of flavonoids and isoflavonoids, are also known as benzalacetophenone or benzylidene acetophenone. They play an important role in plants for the pigmentation flowers, and act against pathogens and insects (Batovska and Todorova, 2010). Studies with chalcones and their analogues have shown several biological activities such as anti-inflammatory, antimicrobial, antioxidant and anticancer activities (Table 2) (Hijova, 2006; Perjési and Rozmer, 2011). A previous study showed that diets containing 500 ppm of chalcone and 2-hydroxychalcone showed an inhibitory effect on oral carcinogenesis induced using 4-nitroquinoline-1-oxide in F344 rats (Makita et al., 1996). Another study showed that chalcones had a preventive effect on diabetic complications. The effect of several chalcones at a dose of 25 mg/kg on glycogen contents in rats showed marked inhibition of aldose reductase, which affected the rate of glycogenolysis by decreasing the glycogen content of the liver (Jamal et al., 2009). These data indicate the importance

of chalcones and their analogues as an emerging class of preventive and therapeutic agents for diabetes.

FLAVONOIDS

Flavonoids are a class of polyphenolic compounds synthesized in plants. In general, flavonoids are compounds characterized by three rings; two aromatic rings linked to one heterocyclic ring. Flavonoids can be divided into flavonols, flavones, flavanones, isoflavonoids, catechins and anthocyanins (Hollman and Katan, 1997; Peterson and Dwyer, 1998). These compounds generally are considered safe because of favorable pharmacological activity and low toxicity (Table 2). Many compounds possess anti-inflammatory, antioxidant, anticarcinogenic and antiallergenic activities (Khairullina et al., 2010). In addition, the antiproliferative effect of the analogue 4'-chloroflavanone was observed in MCF-7 and MDA-MB-453 cells. The synthetic flavanone at a concentration of > 50 $\mu\text{mol/L}$ inhibits cell proliferation through disruption of the G1/S phase and by induction of apoptosis (Choi et al., 2010). Additional biological activities were described for compounds belonging to the class of flavonoids. Recent studies with flavanone compounds showed antihyperglycemic action and an effect on bone metabolism. In an experimental model of diabetes, the flavanone naringenin (50 mg/kg) significantly decreased the levels of fasting blood glucose and glycosylated hemoglobin and increased the serum insulin levels. The same study showed an increase in the activities of the pancreatic enzymatic antioxidants and plasma non-enzymatic antioxidants (Annadurai et al., 2012). Another study showed favorable effects of naringenin and hesperidin on bone metabolism. Naringenin 0.5%, hesperidin 0.5% and naringenin 0.25% + hesperidin 0.25% were administered to male gonad-intact senescent rats, and systemic parameters of bone metabolism were evaluated. The results showed that the flavanones exerted a protective effect on the bone in senescent male rats through inhibition of bone resorption (Habauzit et al., 2011).

In vitro assays showed that the antioxidant capacity of naringenin was higher than that of its glycoside naringin (Cavia-Saiz et al., 2010). In addition, a study on lipid peroxidation in mouse liver homogenates showed that both glycosides naringin and hesperidin had no antioxidant activity. On the other hand, quercetin at a concentration of 1 $\mu\text{mol/L}$ showed antioxidant action (Day et al., 2000). Quercetin, the most abundant flavonoid, is recognized for its antioxidant action; however, several studies have also shown that quercetin has prooxidant activity (Constantin and Bracht, 2008). Moreover, similar to other flavonoids, quercetin showed reversible inhibition of butyrylcholinesterase, an enzyme linked to scavenging (Katalinić et al., 2010).

Studies with harpalycin 2 isoflavone on enzymatic, edematogenic and myotoxic activities of secretory

phospholipase A2 (sPLA2) isolated from *B. pirajai* (Viperidae), *Crotalus durissus* (Viperidae), *Apis mellifera* (Apidae) and *Naja naja* (Elapidae) venoms showed inhibition of all sPLA2s tested, edema and myotoxic activity. These results suggest that harpalycin 2 possesses anti-inflammatory activity mainly in disorders that involve sPLA2 (Ximenes et al., 2012).

CONCLUSION

Plants extracts have been used for the treatment of several human diseases. Although few studies have been performed using *H. brasiliensis*; studies show that this plant represents a particularly rich source of pharmacologically active compounds and possesses more than one bioactivity. This plant has anti-inflammatory, antivenom, antimicrobial, anticarcinogenic and antioxidant activities. Finally, further studies should be performed to identify the clinical applications of this plant for the benefit of mankind.

Conflict of Interest

Authors declare no conflict of interest.

REFERENCES

- Annadurai T, Muralidharan AR, Joseph T, Hsu MJ, Thomas PA, Geraldine P (2012). Antihyperglycemic and antioxidant effects of a flavanone, naringenin, in streptozotocin-nicotinamide-induced experimental. *J. Physiol. Biochem.* 68(3):307-318.
- Aratanechemuge Y, Hibasami H, Katsuzaki H, Imai K, Komiya T (2004). Induction of apoptosis by maackiain and trifolirhizin (maackiain glycoside) isolated from sanzukon (*Sophora Subprostrate* Chen et T. Chen) in human promyelocytic leukemia HL-60 cells. *Oncol. Rep.* 12:1183-1188.
- Araújo RM, Pinheiro SM, Lima MAS, Silveira ER (2008). Complete NMR data assignments for novel pterocarpan form *Harpalyce brasiliensis*. *Magn. Reson. Chem.* 46:890-893.
- Batovska DI, Todorova IT (2010). Trends in utilization of the pharmacological potential of chalcones. *Curr. Clin. Pharmacol.* 5(1):1-29.
- Bhargavan B, Singh D, Gautam AK, Mishra JS, Kumar A, Goel A, Dixit M, Pandey R, Manickavasagam L, Dwivedi SD, Chakravarti B, Jain GK, Ramachandran R, Maurya R, Triverdi A, Chattopadhyay N, Sanyal S (2012). Medicarpin, a legume phytoalexin, stimulates osteoblast differentiation and promotes peak bone mass achievement in rats: evidence for estrogen receptor β -mediated osteogenic action of medicarpin. *J. Nutr. Biochem.* 23(1):27-38.
- Bishayee A, Ahmed S, Brankov N, Perloff M (2011). Triterpenoids as potential agents for the chemoprevention and therapy of breast cancer. *Front. Biosc.* 16:980-996.
- Cavia-Saiz M, Busto MD, Pilar-Izquierdo MC, Ortega N, Perez-Mateos M, Muñoz P (2010). Antioxidant properties, radical scavenging activity and biomolecule protection capacity of flavonoid naringenin and its glycoside naringin: a comparative study. *J. Sci. Food Agr.* 90(7):1238-1244.
- Chintharlapalli S, Papineni S, Lei P, Pathi S, Safe S (2011). Betulinic acid inhibits colon cancer cell and tumor growth and induces proteasome dependent and -independent downregulation of specificity proteins (Sp) transcription factors. *Cancer* 11:371-1/12.
- Chintharlapalli S, Papineni S, Ramaiah SK, Safe S (2007). Betulinic acid inhibits prostate cancer growth through inhibition of specificity

- protein transcription factors. *Cancer Res.* 67(6):2816-2823.
- Choi EJ, Lee JI, Kim GH (2010). Anti-carcinogenic effect of a new analogue 4'-chloroflavanone from flavanone in human breast cancer cells. *Int. J. Mol. Med.* 25(2):293-298.
- Constantin J, Bracht A (2008). Quercetin, mechanisms of anti- and prooxidant activities. *Phytopharmacol. Ther. Values* 21:39-61.
- Darko LL (1984). Method of treating mammals for effects of neuro- and cardiovascular toxins. United States Patent. Patent No. 4,443,472.
- Day AJ, Bao Y, Morgan MRA, Williamson G (2000). Conjugation position of quercetin glucuronides and effect on biological activity. *Free Rad. Biol. Med.* 29:1234-1243.
- Dey A, De JN (2012). Phytopharmacology of antiophidian botanicals: a review. *Int. J. Pharmacol.* 8(2):62-79.
- Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem.* 97:654-660.
- Friedman FK, West D, Dewich PM, Gelboin HV (1985). Specificity of medicarpin and related isoflavonoids in inhibition of rat hepatic mixed function oxidase activity. *Pharmacology* 31(5):289-293.
- Gelboin HV, West D, Gozukara E, Natori S, Nagao M, Sugimura T (1981). (-) Maackiain acetate specifically inhibits different forms of aryl hydrocarbon hydroxylase in rat and man. *Nature* 291:659-661.
- Habauzit V, Sacco SM, Gil-Izquierdo A, Trzeciakiewicz A, Morand C, Barron D, Pinaud S, Offord E, Horcajada MN (2011). Differential effects of two citrus flavanones on bone quality in senescent male rats in relation to their bioavailability and metabolism. *Bones* 49:1108-1116.
- Harinantenaina L, Brodie PJ, Slobodnick C, Callmander MW, Rakotobe E, Randrianasolo S, Randrianaivo R, Rasamison VE, TenDyke K, Shen Y, Suh EM, Kingston DGI (2010). Antiproliferative compounds from *Pongamiopsis pervilleana* from the Madagascar dry forest. *J. Nat. Prod.* 73(9):1559-1562.
- Hijova E (2006). Bioavailability of chalcones. *Bratisl. Lek. Listy.* 107(3):80-84.
- Hollman PCH, Katan MB (1997). Absorption, metabolism and health effects of dietary flavonoids in man. *Biomed. Pharmacother.* 51:305-310.
- Ishiguro M, Tatsuoka T, Nakatsuka N (1982). Synthesis of (+)-cabeneprins A-I and A-II. *Tetrahedron Lett.* 23(38):3859-3862.
- Jamal H, Ansari WH, Rizvi SJ (2009). Chalcones: differential effects on glycongen contents of liver, brain and spinal cord in rats. *Biol. Med.* 1(2):107-115.
- Jiménez-González L, Álvarez-Corral M, Muñoz-Dorado M, Rodríguez-García I (2008). Pterocarpan: interesting natural products with antifungal activity and other biological properties. *Phytochem. Rev.* 7:125-154.
- Katalinić M, Rusak G, Barović JD, Sinko G, Jelić D, Antolović R, Kovarik Z (2010). Structural aspects of flavonoids as inhibitors of human butyrylcholinesterase. *Eur. J. Med. Chem.* 45(1):186-192.
- Khairullina VR, Gerchikov A Ya, Denisova SB (2010). Comparative study of the antioxidant properties of selected flavonols and flavanones. *Kinet. Catal.* 51(2):219-224.
- Krishna PR, Alivelu M (2011). Total synthesis of leiocarpin C and (+)-goniodiol via an olefin cross-metathesis protocol. *Helvetica* 94:1102-1107.
- Makita K, Tanaka T, Fujitsuka H, Tatematsu N, Satoh K, Hara A, Mori H (1996). Chemoprevention of 4-nitroquinoline 1-oxide-induced rat oral carcinogenesis by the dietary flavonoids chalcone, 2-hydroxychalcone, and quercetin. *Cancer Res.* 56:4904-4909.
- Milião GCG, Pinheiro S, Dantas INF, Pessoa C, Moraes MO, Costa-Lotufo LV, Lima MAS, Silveira ER (2007). Biosay-guided fractionation of pterocarpan from roots of *Harpalyce brasiliensis* Benth. *Bioorg. Med. Chem.* 15:6687-6691.
- Nakagawa M, Nakanishi K, Darko LL, Vick JA (1982). Structures of cabeneprins A-I and A-II, potent anti-snake venoms. *Tetrahedron Lett.* 23(38):3855-3858.
- Perjési P, Rozmer Z (2011). Kinetic analysis of some chalcones and synthetic chalcone analogues on the Fenton-reaction initiated deoxyribose degradation assay. *Open Med. Chem. J.* 5:61-67.
- Peterson J, Dwyer J (1998). Flavonoids: dietary occurrence and biochemical activity. *Nutr. Res.* 18:1995-2018.
- Silva AJM, Coelho AL, Simas ABC, Moraes RAM, Pinheiro DA, Fernandes FFA, Arruda EZ, Costa PRR, Melo PA (2004). Synthesis and pharmacological evaluation of prenylated and benzylated pterocarpan against snake venom. *Bioorg. Med. Chem. Lett.* 14:431-435.
- Silva GL, Machado MIL, Matos FJA, Braz-Filho R (1999). A new isoflavone isolated from *Harpalyce brasiliensis*. *J. Braz. Chem. Soc.* 10(6):438-442.
- Silva GL, Matos FJA, Silveira ER (1997). 4'-dehydroxycabeneprin A-I from roots *Harpalyce brasiliensis*. *Phytochemistry* 46(6):1059-1062.
- Skinninger L, Stoessl A (1986). The effect of the phytoalexins, lubimin, (-)-maackiain, pinosylvin, and the related compounds dehydroloroglossol and hordatine M on human lymphoblastoid cell lines I. *Experientia* 42:568-570.
- Tyagi AM, Gautam AK, Kumar A, Srivastava K, Bhargavan B, Trivedi R, Saravanan S, Yadav DK, Singh N, Pollet C, Brazier M, Mentaverri R, Maurya R, Chattopadhyay N, Goel A, Singh D (2010). Medicarpin inhibits osteoclastogenesis and has nonestrogenic bone conserving effect in ovariectomized mice. *Mol. Cell. Endocrinol.* 325(1-2):101-109 DOI: 10.1016/j.mce.2010.05.016.
- Van Aardt TG, Van Resburg H, Ferreira D (2001). Synthesis of isoflavonoids. Enantiopure cis and trans-6a-hydroxypterocarpan and racemic trans-pterocarpan. *Tetrahedron* 57:7113-7126.
- Vieira NC, Espíndola LS, Santana JM, Veras ML, Pessoa ODL, Pinheiro SM, Araújo RM, Lima MAS, Silveira ER (2008). Trypanocidal activity of a new pterocarpan and other secondary metabolites of plants from Northeastern Brazil flora. *Bioorg. Med. Chem.* 16:1676-1682.
- Ximenes RM, Rabello MM, Araújo RM, Silveira ER, Fagundes FHR, Diz-Filho EBS, Buzzo SC, Soares VCG, Toyama DO, Gaeta HH, Hernandes MZ, Monteiro HAS, Toyama MH (2012). Inhibition of neurotoxic secretory phospholipases A2 enzymatic, edematogenic, and myotoxic activities by harpalycin 2, an isoflavone isolated from *Harpalyce brasiliensis* Benth. *Evid-Based Compl. Alt.*, Article ID 987517. doi: 10.1155/2012/987517.
- Xu R, Fazio GC, Matsuda SPT (2004). On the origins of triterpenoid skeletal diversity. *Phytochemistry* 65(3):261-291.
- Yadav JS, Premalatha K, Harshavardhan SJ, Subba Reddy BV (2008). The first stereoselective and the total synthesis of leiocarpin C and total synthesis of (+)-goniodiol. *Tetrahedron Lett.* 49:6765-6767.
- Zhou H, Lutterodt H, Cheng Z, Yu L(L) (2009). Anti-inflammatory and antiproliferative activities of trifolirhizin, a flavonoid from *Sophora flavescens* roots. *J. Agric. Food Chem.* 57(11):4580-4585.

Full Length Research Paper

Andiroba oil (*Carapa guianensis*) on ventral hernia repair

Edson Yuzur Yasojima^{1*}, Felipe Lobato da Silva Costa², Abdallah de Paula Houat², Renan Kleber Costa Teixeira², Edvaldo Lima Silveira³, Marcus Vinicius Henriques Brito² and Gaspar de Jesus Lopes Filho¹

¹Division of Gastroenterology Surgery, University of Sao Paulo, Brazil

²Para State University, Brazil.

³Infectious and Parasitic Diseases, University of Para, Brazil.

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In the Amazon rainforest region, Andiroba oil is an herbal oil that is widely used by the local population to treat several inflammatory diseases. The objective of this study was to test Andiroba oil as a mesh coating or by oral administration in a rat model of excisional abdominal wall defect as an alternative to modulate inflammatory response without impairing the ventral hernia repair. Thirty six animals were distributed into three groups (N=12). (1) In the control group (CONT), ventral hernia repair was done using polypropylene/polyglecaprone mesh. (2) In the Andiroba oil gavage group (AndG), animals were treated with the meshes and 0.63 ml/kg of Andiroba oil was given by oral administration for 7 days prior to the mesh placement. (3) In the Andiroba oil submersion group (AndS), animals were treated with meshes that were previously submersed in Andiroba oil. At the 7th, 14th, and 21st days, macroscopic and microscopic analyses were done. AndG had fewer adhesions, necrosis, and lymphocytes, as well as similar collagen fiber formation and fibrosis areas as CONT. AndS showed a higher number of macrophages, fibrosis area, and less collagen fiber formation. Oral administration of Andiroba oil modulated inflammatory response, reduced abdominal adhesion formation, and did not impair tissue healing.

Key words: Abdominal wall, wound healing, Andiroba, herbal, cicatrization.

INTRODUCTION

Incisional hernia is one of the most frequent complications of laparotomy; its incidence varies between 2 and 20% after all abdominal surgeries (Burger et al., 2006). However, actual incidence might be higher than the reported data, because most patients with incisional hernias

have no symptoms. Hernia recurrence is one of the most undesirable complications, needing a complicated repair surgery and demanding high costs (Franklin et al., 2004). In the United States, the total number of ventral hernia repair (VHR) surgeries has increased substantially in the

*Corresponding author. E-mail: yasojima@qlobo.com. Tel: +55 91 9991-8882

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past few years, having an annual cost of 3.2 billion dollars (Poulouse et al., 2012).

Incisional hernia treatment is essentially surgical and there are several techniques for their correction, as for example, primary suture of the wall (Baroncello et al., 2008). However, the best results are obtained through mesh reinforcement, in which the chances of recurrence vary between 3 and 17%. It is clear that the use of meshes has revolutionized the outcomes of VHR (Montgomery, 2013).

Several mesh types have been widely used in surgical practice since their development. Meshes can be non-absorbable, absorbable, or partially absorbable; the last two being more commonly used (Pundek et al., 2010). Polypropylene, polyester, and ePTFE are the most commonly used meshes, and they also exhibit different properties. The development of new synthetic meshes is also extensive and fast (Montgomery, 2013).

The foreign body reaction is minimized by changing from heavy-weight "standard" pore meshes to low-weight "mega" pore meshes, which might reduce the fibrotic scar tissue formation and shrinkage around the mesh (Klinge et al., 2012). Polypropylene mesh exhibits mega pores, good tensile strength, adequate inflammatory response, and low fibrosis, and is being widely used (Van'tRiet et al., 2003; Butler et al., 2004).

Retromuscular position seems to be the most commonly used and safe mesh placement site, and the mesh must be ideally separated from abdominal organs in order to avoid adhesion formation (Montgomery, 2013). However, in large abdominal wall defects, it is very difficult to avoid contact between abdominal organs and the mesh, leading to an increased number of adhesions and other complications such as fistula formation and intestinal obstruction (Dinsmore et al., 2000; Alimoglu et al., 2003).

Polypropylene mesh can induce dense abdominal adhesions to peritoneal structures; however, adhesion avoidance is desirable. Synthetic meshes are usually designed to be positioned outside the abdominal cavity, unless a special coating has been applied for intra-abdominal use (Montgomery, 2013). Several substances were tested, but there is not yet an ideal mesh coating that would show no adverse reactions, complications, or foreign body reaction that would be simple to use, easily accessible, and have a low cost (Toosie et al., 2000; Lontra et al., 2010; Zong et al., 2004).

In the Amazon rainforest region, Andiroba oil is an herbal extract widely used by the local population to treat several inflammatory diseases. Today, the level of public policies of the World Health Organization encourages the use of medicinal plants. Because Andiroba oil has proven to have anti-inflammatory and healing effects (Brito et al., 2001; Rodrigues et al., 2008; Brito et al., 2006), it could be of great use as a mesh coating or therapeutic drug for VHR. Thus, Andiroba oil was tested as a mesh coating or by oral administration, in a VHR model of abdominal wall

defect, to improve abdominal wall repair.

MATERIALS AND METHODS

Animals

Thirty-six male Wistar rats (15 to 20 weeks), weighting 250 to 300 g, were used in this study. The animals were kept in a vivarium of the Laboratory for Experimental Surgery at Para State University (Brazil) in a temperature-, light-, humidity-, and noise-controlled environment. Water and food were provided *ad libitum*. This research strictly followed the rules of the Brazilian National Law for Animal Care (Law: 11.794/08) that is based on NIH guidelines, and followed the rules of Council for International Organization of Medical Sciences ethical code for animal experimentation. The project was previously approved by the animal use and care committee at Para State University (AUCC-UEPA).

Experimental protocol

The animals were randomly assigned into the following three groups ($n=12$ for each group): (1) In the control group (CONT), VHR was done using only polypropylene/polyglycolic meshes. (2) In the Andiroba oil gavage group (AndO), animals were treated with meshes and 0.63 ml/kg Andiroba oil by oral administration, 7 days prior to the mesh placement. (3) In the Andiroba oil submersion group (AndS), animals were treated with meshes that were previously submersed in Andiroba oil for 15 min immediately prior to their placement.

Surgical procedures

All procedures were performed in anesthesia (ketamine hydrochloride and xylazine hydrochloride 60 and 6 mg/kg, respectively, i.p.).

Crude *Carapa guianensis* oleoresin oil was obtained from the Brazilian National Laboratory for Research in Medicinal Plants. In the AndG group, oral administration was given by gavage directly into the stomach, once in a day, for 7 consecutive days prior to the surgery. The administered daily dose was 0.63 ml/kg. Such dose was previously used by Rodrigues et al. (2008), who analyzed different doses of Andiroba oil and showed that at 0.63 ml/kg there was systemic inflammatory response modulation after a kidney injury and no toxicity to other organs.

In all animals, laparotomy was performed using a 4-cm skin incision at the midline and the exposure of the aponeurotic muscle layer. Immediately after, an excision was done on the ventral part of the abdomen, involving the aponeurotic muscle layer and the peritoneum, with a 2-cm longitudinal axis and 2-cm transversal axis, in order to create a ventral defect in the aponeurotic muscle.

Ventral hernia was repaired in all groups by the placement of polypropylene/polyglycolic meshes having 3-cm longitudinal and transversal axes, attached at the edges with eight separated stitches (6-0 nylon thread), equidistant, performing five semi-knots in each stitch and leaving the mesh margins over the anterior aponeurotic plane (Figure 1).

Animals were euthanized by lethal anesthetic doses at three time points (4 animals each group): 7, 14, and 21 days. Meshes were found through the incision that was done previously. Then macroscopic analysis was performed regarding the presence of incisional hernias, infections, dehiscence or fistulas and the total number of adhesions. After this, scar tissues above and nearby meshes and the entire mesh were harvested for histological analysis.

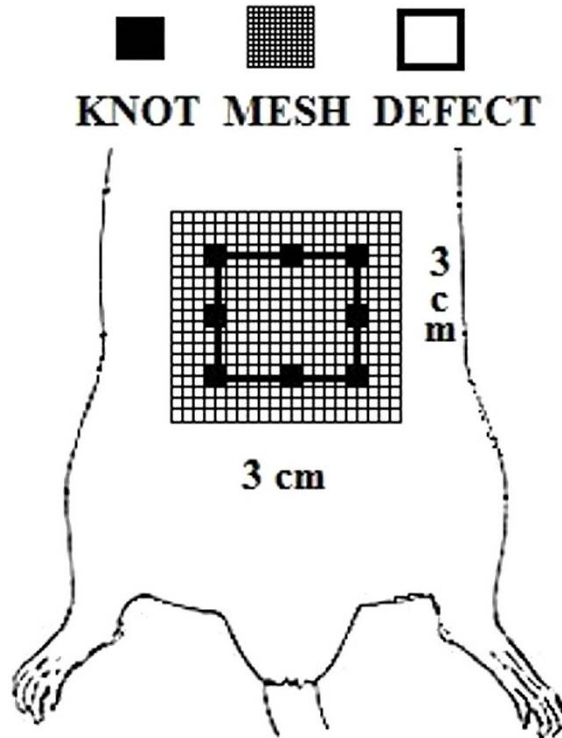


Figure 1. Surgical protocol.

Histological examination

Harvested tissue was fixed in 10% buffered formaldehyde solution, embedded in paraffin, and stained using hematoxyline/eosin and Masson's trichrome.

At the hematoxyline/eosin stain, inflammatory response parameters were analyzed. Macrophage, lymphocyte, and giant cell counting around mesh fragments were performed and classified as 0=absence, 1=mild, 2=moderate, and 3=intense. Necrosis, fibrosis, and type of granuloma were classified as 0=absence, 1=mild, 2=moderate, and 3=intense.

At Masson's trichrome stain, collagen fibers were quantified and classified as 0=absence, 1=mild, 2=moderate, and 3=intense.

Statistics

Normal distribution of data was confirmed using the Kolmogorov-Smirnov test. Results were analyzed by Kruskal-Wallis test. Statistical significance was assumed at $p < 0.05$.

RESULTS

Macroscopic analysis

Incisional hernias, infections signs, dehiscence, or fistula formation were not detected in any animal. Abdominal adhesions between abdominal organs and the mesh were detected in all animals; however, AndG animals showed fewer adhesions than CONT at the 14th and 21st days (Table 1).

Inflammatory response

All animals showed acute inflammatory response that was characterized by swelling, vascular congestion, and neutrophil infiltration. Regarding other immunologic cell counting, AndS showed a higher number of macrophages than AndG in all three time points, whereas CONT showed higher giant cells and lymphocytes counting than AndS and AndG in all three time points (Table 2).

Granulomas formed around the meshes were histologically characterized as a foreign body type response granuloma, and were composed of macrophages and giant cells around each mesh fragment observed, showing no difference on the intensity among groups.

Necrosis was observed in all animals; however, AndG showed less necrotic areas than CONT and AndS in all three time points. Fibrotic areas appeared only at the 21st day on CONT, but have been identified since the 7th day on AndG and AndS. AndS showed higher degrees of fibrosis in all three time points and AndG showed a fibrosis degree similar to CONT at the 21st day (Table 3).

Collagen synthesis

CONT and AndG showed increased collagen fibers within postoperative days; however, AndS showed a rapid increase during the first postoperative week and subsequent stabilization of collagen fibers in growth. AndG showed a faster ingrowth of collagen fibers than CONT, reaching its peak on Day 14 (Figure 2).

DISCUSSION

After abdominal wall surgeries, postoperative pain, which is a major patient complaint, can be diminished using non steroidal anti-inflammatory drugs; however, these drugs can slow down the healing process and increase the chances of complications (Alimoglu et al., 2003). Some anti-inflammatory drugs have already been tested on VHR, and the results remain controversial. Andiroba oil shows anti-inflammatory, wound healing, and antimicrobial actions, thus it could be of great application for a better VHR outcome as it could modulate systemic inflammatory response, avoid infections, and lead to a better wound healing outcome (Brito et al., 2001; Rodrigues et al., 2008; Brito et al., 2006; Santos et al., 2012).

The absence of dehiscence, fistula formation, or infections in all animals demonstrates that Andiroba oil did not strongly interfere in the early abdominal wall healing process; early changes occurred in studies using parecoxibe (Kyriakidis et al., 2011) and meloxicam (Tognini, et al., 2000). Macroscopic results were similar to those found by Pundek et al. (2010), who analyzed that VHR was analyzed when only polypropylene/polyglycaprone meshes were used.

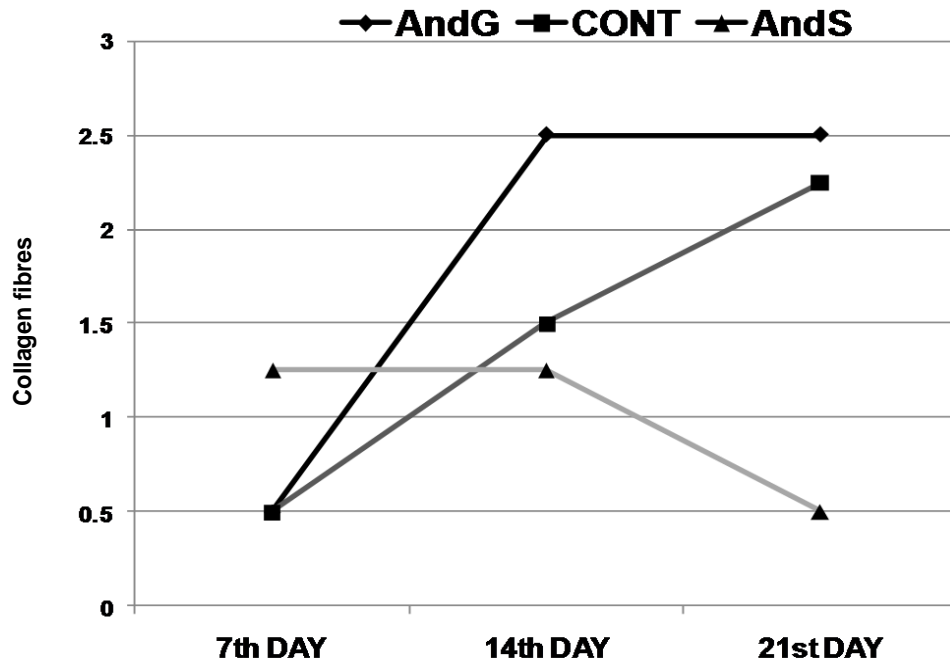


Figure 2. Collagen fibers within days. $P < 0.05$ AndG vs. CONT at 14th day; AndG vs. AndS at 21st day.

Table 1. Average number of adhesions between the mesh and abdominal organs.

Group	CONT	AndO	AndS
7th day	2.75	1.75	2
14th day	3*	1.5*	2.5
21st day	3.75*	1.5*	2.75

*Statistical difference; $p < 0.05$. CONT vs. AndG at 14 and 21 days (Kruskal-Wallis test).

Table 2. Groups average of inflammatory cell counting surrounding mesh fragments.

Group	CONT	AndG	AndS
Macrophages*			
7th day	2	1	2
14th day	2	1	2
21st day	2	1	2
Giant cells[#]			
7th day	3	2	2
14th day	3	2	2
21st day	3	2	2
Lymphocytes[#]			
7th day	3	2	2
14th day	3	2	2
21st day	3	2	2

* $p < 0.05$ AndG vs. CONT and AndS (Kruskal-Wallis test); [#] $p < 0.05$ CONT vs. AndG and AndS (Kruskal-Wallis test).

Table 3. Average of necrosis and fibrosis area among groups.

Group	CONT		AndG		AndS	
	Necrosis*	Fibrosis	Necrosis*	Fibrosis [#]	Necrosis	Fibrosis [#]
7th day	3	0	1	1	1.75	3
14th day	2.25	0	1	1	2.25	3
21th day	3	0.75	1	1	1.5	3

*p < 0.05 AndG vs. CONT (Kruskal-Wallis test); [#]p < 0.05 AndS vs. AndO (Kruskal-Wallis test).

Abdominal adhesions can occur in up to 90 to 100% of all abdominal surgeries and can lead to severe complications, such as fistula formation and intestinal obstruction (Buerger et al., 2006). Almost all meshes may produce adhesions when they are in contact with the intestinal surface, and this process is determined by the size of the pores and the structure of the mesh (Klinge et al., 2012).

Oral administration of Andiroba oil was able to reduce the number of adhesions between the mesh and the abdominal organs when compared with CONT (Table 1). This fact probably occurred due to modulation of the inflammatory response by Andiroba oil, reducing aggression between the parietal and visceral serosa, and thereby reducing the formation of adhesions (Ricciardi et al., 2012).

When Andiroba oil was used as a mesh coating, abdominal adhesions were similar to CONT. It occurred probably, because Andiroba oil was not systemically absorbed and acted as a local chemical irritant, contributing to adhesion formation (Souza Junior et al., 1999). This fact was confirmed by higher macrophage counting on the AndS group, showing a greater foreign body type response.

A reduced number of lymphocytes and giant cells were the reason for less local inflammatory response in both Andiroba groups (Table 2) as the lymphocytes are responsible for inflammatory response augmentation (Ferrante et al., 2012).

Necrotic areas occurred due to foreign body type inflammatory response. All meshes will introduce a foreign body type response that needs to be balanced in order to result in normal wound healing. Chemicals can remain within the mesh, causing both toxic and inflammatory reactions in the patient (Montgomery, 2013). AndG was the only group that showed reduced necrosis areas in all three time points analyzed, confirming that oral administration of Andiroba oil can modulate the inflammatory response secondary to VHR.

During VHR, there are many steps that can fail; early degradation or absorption of the mesh and fibrosis formation are undesirable (Montgomery, 2013). Andiroba oil inflammatory response modulation did not significantly interfere with fibrosis formation, which was similar in both AndG and CONT groups (Table 3). Albeit, when the herbal extract was used as a mesh coating, there were more intense fibrosis and mesh reabsorption, probably due to the greater foreign body type response (Ferrante

et al., 2012).

When collagen fiber ingrowth was analyzed (Figure 2), AndG and CONT groups had the same final amount of collagen fibers deposited on the mesh; however, oral administration of Andiroba oil accelerated collagen fiber formation, reaching its peak one week before the control group. On the other hand, when Andiroba oil as a mesh coating was tested, there was less collagen fibers ingrowth when compared with the other groups, exposing that there was worse tissue healing.

There is unceasing search for an ideal mesh coating that would show no adverse reactions, complications, or foreign body reaction, and one that is simple to use, easily accessible, and affordable (Montgomery, 2013). However, this study shows that no substance, despite its anti-inflammatory or tissue healing activity, should be used in the clinical setting of VRH before a full basic research is done, because Andiroba oil used as a mesh coating showed greater fibrosis and less collagen fiber formation, leading to worse VHR outcome.

On the other hand, oral administration of Andiroba oil showed promising results on this adopted experimental setting of VHR. It was able to modulate the systemic inflammatory response, reducing abdominal adhesion formation; interestingly, it did not diminish tissue healing, had low ratio of fibrosis, and had a faster and satisfactory collagen fiber formation. This fact shows that there is a wide field of study for the development of new medicinal plant-related drugs that could be used in the clinical setting of VHR.

Conclusion

Oral administration of Andiroba oil modulated inflammatory response, reducing abdominal adhesion formation; interestingly, it did not diminish tissue healing, had low ratio of fibrosis, and had a faster and satisfactory collagen fiber formation, delivering a better VHR outcome. However, when it was used as a mesh coating, greater fibrosis and less collagen fibers formation were seen, exposing worse VHR outcome.

ABBREVIATIONS

VHR, Ventral hernia repair; **AndG**, Andiroba oil gavage group; **AndS**, Andiroba oil submersion group; **CONT**, control group.

Conflict of Interest

Authors declare no conflict of interest.

REFERENCES

- Alimoglu O, Akcakaya A, Sahin M, Unlu Y, Ozkan OV, Sanli E, Eryilmaz R (2003). Prevention of adhesion formations following repair of abdominal wall defects with prosthetic materials (an experimental study). *Hepatogastroenterology* 50(51):725-728.
- Baroncello JB, Czeczko NG, Malafaia O, Ribas-Filho JM, Nassif PAN, Dietz AU (2008). The repair of abdominal defects in rabbits with Parietex® and Surgisis® meshes abdominal wall. *Arq. Gastroenterol.* 45(4): 323-329.
- Brito MVH, Figueiredo RC, Tavares MLC, Silveira TS, Cantanhêde G (2006). Andiroba and copaiba oils effect in induced miositis in rats. *Rev. Para. Med.* 20(2):17-24.
- Brito NMB, Silva PRFS, Silva GCF, Casella SFM, Sampaio ARS, Carvalho RA (2001). Macroscopic evaluation of the cicatrization of treated open cutaneous wounds with oil andiroba in rats. *Rev. Para. Med.* 15(2): 17-22.
- Burger JWA, Halm JA, Wijsmuller AR, Raa ST, Jeekel J (2006). Evaluation of new prosthetic meshes for ventral hernia repair. *Surg. Endosc.* 20(8):1320-1325.
- Butler CE, Prieto VG (2004). Reduction of adhesions with composite AlloDerm/polypropylene mesh implants for abdominal wall reconstruction. *Plast. Reconstr. Surg.* 114(2):464-473.
- Dinsmore RC, Calton Junior WC, Harvey SB, Blaney MW (2000). Prevention of adhesions to polypropylene mesh in a traumatized bowel model. *J. Am. Coll. Surg.* 191(2):131-136.
- Ferrante CJ, Leibovich SJ (2012). Regulation of macrophage polarization and wound healing. *Adv. Wound Care* 1(1):10-16.
- Franklin ME, Gonzalez JJ, Glass JL, Manjarrez A (2004). Laparoscopic ventral and incisional hernia repair: an 11-year experience. *Hernia* 8(1):23-27.
- Klinge U, Klosterhalfen B (2012). Modified classification of surgical meshes for hernia repair based on the analyses of 1,000 explanted meshes. *Hernia* 16(3):251-258.
- Kyriakidis AV, Perysinakis I, Alexandris I, Athanasiou K, Papadopoulos C, Mpesikos I (2011). Parecoxib sodium in the treatment of postoperative pain after lichtenstein tension-free mesh inguinal hernia repair. *Hernia* 15(1):59-64.
- Lontra MB, Bigolin AV, Costa RG, Grossi JV, Scalco P, Roll S, Cavazzola LT (2010). Effectiveness of the combined use of lactic acid film and polypropylene mesh in the formation of intraperitoneal adhesions: an experimental model in rats. *Rev. Col. Bras. Cir.* 37(5):364-369.
- Montgomery A (2013). The battle between biological and synthetic meshes in ventral hernia repair. *Hernia* 17(1):3-11.
- Poulose BK, Shelton J, Phillips S, Moore D, Nealon W, Penso S, Holzman MD (2012). Epidemiology and cost of ventral hernia repair: making the case for hernia research. *Hernia* 16(2):179-183
- Pundek MRZ, Czeczko NG, Yamamoto CT, Pizzatto RF, Czeczko LEA, Dietz UA, Malafaia O (2010). Study of surgical meshes of polypropylene/polyglycaprone and polypropylene/ polydioxanone/ oxidized regenerated cellulose on the healing of defects produced in abdominal wall of rats. *Arq. Bras. Cir. Dig.* 23(2):94-99.
- Ricciardi BF, Chequim LH, Gama RR, Hasegawa L (2012). Abdominal hernia repair with mesh surrounded by fibrous tissue - experimental study in Wistar rats. *Rev. Col. Bras. Cir.* 39(3):195-200.
- Rodrigues BD, Fonseca, AXC, Brito MVH, Brito NMB, Brito RB (2008). Effect of andiroba oil in of rats submitted to renal ischemia and Reperfusion. *Rev. Para. Med.* 22(2):9-16.
- Santos RC, dos Santos Alves CF, Schneider T, Lopes LQ, Aurich C, Giongo JL, Brandelli A, de Almeida Vaucher R (2012). Antimicrobial activity of Amazonian oils against *Paenibacillus* species. *J. Invertebr. Pathol.* 109(3):265-268.
- Souza Jr OG, Cal RVR, Amoury Jr RRC, Rocha ABMA (1999). Effect of andiroba oil in the peritoneal cavity of rats. *Rev. Para. Med.* 13(2):47-50.
- Tognini JRF, Fagundes DJ, Novo NF, Juliano Y (2000). Biomechanical and morphological study in rats'abdominal wall healing under meloxican action. *Acta Cir. Bras.* 15(3):10-15.
- Toosie K, Gallego K, Stabile BE, Schaber B, French S, de Virgilio C (2000). Fibrin glue reduces intra-abdominal adhesions to synthetic mesh in a rat ventral hernia model. *Am. Surg.* 66(1):41-45.
- Van 'tRiet M, de Vos van Steenwijk PJ, Bonthuis F, Marquet RL, Steyerberg EW, Jeekel J, Bonjer HJ (2003). Prevention of adhesion to prosthetic mesh: comparison of different barriers using an incisional hernia model. *Ann. Surg.* 237(1):123-128.
- Zong X, Li S, Chen E, Garlick B, Kim KS, Fang D, Chiu J, Zimmerman T, Brathwaite C, Hsiao BS, Chu B (2004). Prevention of postsurgery-induced abdominal adhesions by electrospunbioabsorbablenanofibrouspoly(lactide-co-glycolide)-based membranes. *Ann. Surg.* 240(5):910-915.

Full Length Research Paper

DNA damage protecting activity and *in vitro* antioxidant potential of the methanol extract of Cherry (*Prunus avium* L)

Türkan Kutlu^{1*}, Kasim Takim¹, Bircan Çeken² and Murat Kizil²

¹Department of Chemistry, Faculty of Science and Arts, University of Inonu, 44280, Malatya, Turkey.

²Department of Chemistry, Faculty of Science, University of Dicle, 21280, Diyarbakır, Turkey.

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The characterization of sweet cherry (*Prunus avium* L.) genetic resources in Turkey may help to increase their use in breeding programmers worldwide, as Turkey is the centre of origin of sweet cherry. In the Malatya region, cherry leaves are gathered in April and May, fresh and dried used for stuffing. Components of cherry trees have been used as traditional herbal remedies for various diseases. These components are known to possess antioxidative effects. However, the mechanisms underlying cherry tree component-mediated antioxidative effects remain largely unknown. This study focused on cherry 0-900 Ziraat (Malatya Dalbastı) leaves methanol extract (CLME) and examined antioxidant capacity and DNA damage protecting activity. The antioxidant capacity of these extracts were evaluated using different antioxidant tests, including reducing power, free radical scavenging, hydroxyl radical scavenging, hydrogen peroxide scavenging, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ABTS radical scavenging, deoxyribose assay, β -Carotene bleaching assay and metal chelating activities. The content of total phenolic compounds in CLME was determined using Folin-Ciocalteus reagent and compared with standard antioxidants butylated hydroxytoluene (BHT), Trolox and α -tocopherol. CLME showed a concentration-dependent free radical scavenging capacity and protective DNA strand scission by OH[•] on pBR322 super coiled plasmid DNA (95 to 97%). Total phenolic effect on DNA cleavage CLME at 400 μ g/ml exhibited significant protecting activity against the compounds in the CLME and was determined as gallic acid equivalent 132.17 meq/g dried leaves.

Key words: Cherry 0-900 ziraat (Malatya dalbastı), antioxidant activity, radical scavenging ability, DNA damage, phenolic compounds.

INTRODUCTION

The sweet cherry was originated in the mountains of North Eastern part of Turkey, near the Black Sea region, from where it has spread in Roman times (Zohary and

Hopf, 2000). Turkey is one of the leading countries in production and export of sweet cherries (Faostat, 2011). Suitable climatic conditions and excellent export market

*Corresponding author: E-mail: turkan.kutlu@inonu.edu.tr. Tel: +90 422 377 3710. Fax: +90 422 3410037.

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demand have resulted in continuous increase in annual production. *Prunus avium* L is the sweet cherry. Sweet cherry is a vigorous tree with strong apical control with an erect-pyramidal canopy shape; grows to 18 m. In cultivation, sweet cherries are maintained <4 m in height. Leaves are relatively large, elliptic with acute tips, petioles, and strongly veined (Anonymous, 2003). Fruits of these species are not only consumed fresh, but also used to produce jam, jelly, stewed fruit, marmalade, syrup and several types of soft drinks. It is also used for medical purposes due to properties of stalk and fruits. The leaves and seed of these species are used in pharmaceuticals. The tree is also valuable for ornamentation as an evergreen broad leaf plant (Islam, 2002). In addition, stuffed cherry leaves are consumed as food in Malatya.

Free radicals are the molecules with unpaired electrons and commonly called reactive oxygen species (ROS). Free radicals are generated during the process of cellular oxidation, some examples includes superoxide anion, hydrogen peroxide, hydroxyl and nitric oxide radical. These radicals are electrically charged, unstable and highly reactive in nature. It reacts with nucleic acids, mitochondria, proteins and enzymes and resulted in their damage in the cell. However, antioxidant defense system protects the cell from the free radical mediated oxidative stress. When there is over production of free radicals or the failure of an antioxidant defense system, these radicals resulted in tissue injury and cause numerous physiological disorders in the body, cancer, Parkinson's disease, Alzheimer's disease, myocardial infarction and diabetes (Waris and Ahsan, 2006; Zhou et al., 2008; Praticò, 2008; Elahi et al., 2009; Uttara et al., 2009; Wright et al., 2006). It has been proved that a diet rich in antioxidants strengthens the antioxidant defense system and can effectively neutralize the free radicals in body.

Currently, many kinds of synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), and propyl gallate (PG), which are commonly used in processed foods, are known to have toxic and carcinogenic effects on human health. Natural antioxidants, such as α -tocopherol and L-ascorbic acid, are widely used, because they are seen as being safer, but their antioxidant activities are lower than those of synthetic antioxidants (Barlow, 1990). Based on accumulative evidence, in recent decades, tremendous interest has considerably increased in finding natural substances (that is, antioxidants) present in foods or medicinal plants to replace synthetic antioxidants, which are being restricted due to their side effects. On the other hand, polyphenols, used as natural antioxidants, are gaining importance, due to their health benefits for humans, decreasing the risk of cardiovascular and degenerative diseases by reduction of oxidative stress and counteraction of macromolecular oxidation (Bingham et al., 2003; Silva et al., 2004). Thus, much attention has

been focused on the investigation of natural antioxidant compounds from plants, which can effectively scavenge ROS.

Compositional characteristics and antioxidant components, such as oxygen radical absorbance capacity (ORAC) using fluorescein (FL), phenolic acids, and total contents of phenolics, anthocyanins, and carotenoids, of cherry laurel varieties and pekmez were investigated (Alasalvar et al., 2005). However, relatively little or no information is available on free-radical scavenging activities, reducing power, and inhibition of oxidation of human low-density lipoprotein (LDL) cholesterol of cherry laurel fruit and its pekmez (Kolayli et al., 2003).

Cherry laurel fruit and its concentrated juice (pekmez) were examined for their antioxidant activities using different free-radical scavenging activity tests [hydrogen peroxide, superoxide radical, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical], together with reducing power and inhibition of oxidation of human low-density lipoprotein cholesterol. On a fresh weight basis, pekmez exhibited a significantly ($P < 0.01$) higher antioxidant activity than that of cherry laurel fruit in most cases. However, on a dry weight basis hydrogen peroxide and DPPH radical scavenging activities, and reducing power were significantly higher ($P < 0.01$) in cherry laurel fruit than in its pekmez, with some exceptions, thus indicating possible destruction of antioxidative compounds during pekmez production (Ilyana-Pathirana et al., 2006).

There have been only a few studies on the pharmacological effects of cherry leaves. Cherry leaves extract (CLE) was shown to have antioxidant activity through reduction of ROS production that showed a phenolic glucoside isolated from CLE exhibited peroxynitrite scavenging (Jung et al., 2002; Jung et al., 2005). Thus, previous studies *in vitro* showed that CLE has antioxidative effects. However, the mechanisms underlying CLE mediated antioxidative effects remain largely unknown. CLE showed that it prevented t-BOOH-induced reduction of thioredoxin-2 (Trx2), but not thioredoxin-1 (Trx1) and Trx reductases (TrxR1 and TrxR2) protein expression. CLE prevents tert-butyl hydroperoxide (t-BOOH)-induced reduction in (Trx2) expression, promotion of ROS production, activation of p38 kinase, and increase in DNA damage and protects against cell death (Taguchi et al., 2011).

The aim of this study was to investigate the antioxidant and DNA damage protecting activity of a methanol extract of cherry leaves.

MATERIALS AND METHODS

Chemicals

pBR322 super coiled plasmid DNA, agarose, ethidium bromide, Tris-Borat-EDTA gel buffer, bromophenol blue, and EDTA were purchased from Vivantis; ammonium molybdate tetrahydrate, aluminum chloride hexahydrate, sodium nitrite, sodium carbonate

decahydrate, trolox, α -tocopherol, ethanol, methanol, ethyl acetate, DPPH, Tween 20, hydrogen peroxide, linoleic acid, and trans-beta-carotene were purchased from Sigma-Aldrich; chloroform, potassium ferricyanide, sodium thiosulfate, iron chloride tetrahydrate, FeSO_4 , sulphuric acid, β -mercapto ethanol, MnCl_2 , Iron (III) chloride, and sodium salicylate were purchased from Merck; trichloroacetic acid (Alfa Aesar) and BHT were purchased from Safer; methanol, $\text{K}_3\text{Fe}(\text{CN})_6$, ferrozine, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) were purchased from (Fluka), 2-Deoxy-D-Ribose were purchased from Alfa Aesar; potassium dihydrogen phosphate was purchased from Riedel.

Plant and extraction

Sweet cherry cultivars 0-900 Ziraat (Dalbastı) grown in Malatya region of Turkey were used in this study. Cherry leaves were collected from the area of Malatya Fruit Research Institute, Malatya, Turkey in the first week of June, 2009. Leaves were dried for 10 days at room temperature. Cherry leaves (20 g) were ground in an electric blender and then incubated into a glass flask with 300 ml of methanol in the dark for 3 days on a magnetic stirrer, at 25°C, filtered, and concentrated by using a rotary evaporator (İka RV 05 Basic 1B). The crude methanol extract of cherry leaves (1,23 g), as a green color, was obtained in dark glass bottles at 4°C until use.

Determination of total phenolic compounds

The content of total phenolic compounds in the methanol extract of cherry leaves was determined using Folin-Ciocalteus reagent according to the method of Singleton et al. (1999). Crude methanol extract (40 μl) of cherry leaves (1 mg/ml) was mixed with 200 μl Folin-Ciocalteus reagent and 1160 μl of distilled water, followed by 600 μl 20% sodium carbonate (Na_2CO_3) at 3 min later. The mixture was shaken for 2 h at room temperature and absorbance was measured at 765 nm. All tests were performed in triplicate. Gallic acid was used as a standard. The concentration of total phenolic compounds in cherry leaves extracts was determined as a μg of gallic acid equivalents per 1 mg of extract using the following equation obtained from a standard gallic acid graph ($R^2 = 0.9878$):

$$\text{Absorbance} = 0.0012 \times \text{gallic acid } (\mu\text{g}).$$

Scavenging activity of DPPH radical

The free radical scavenging activity of CLME was measured by DPPH using the previously reported procedure (Yen and Chien, 2000). Briefly, 0.1 mM solution of DPPH in methanol was prepared. Then, 1 ml of this solution was added to 3 ml of CLME solution at different concentrations (0.050 to 0.50 mg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, the absorbance was measured at 517 nm in a spectrophotometer (Shimadzu, UV/Visible Recording, Kyoto, Japan). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The radical scavenging activity was calculated as follows:

$$\text{Scavenging effect } (\%) = [(A_{517} \text{ of control} - A_{517} \text{ of sample}) / A_{517} \text{ of control}] \times 100.$$

where BHT, α -tocopherol and Trolox were used as positive controls.

ABTS radical cation decolorization assay

The spectrophotometric analysis of $\text{ABTS}^{+\cdot}$ radical scavenging

activity was determined according to the method of Re et al. (1999). This method is based on the ability of antioxidants to quench the long-lived $\text{ABTS}^{+\cdot}$ radical cation, a blue/green chromophore with characteristic absorption at 734 nm, in comparison to that of BHT, α -tocopherol and trolox, a water-soluble α -tocopherol analogue. The $\text{ABTS}^{+\cdot}$ was produced by reacting 7 mM ABTS in H_2O with 2.45 mM potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$), stored in the dark at room temperature for 12 h. Before usage, the $\text{ABTS}^{+\cdot}$ solution was diluted to get an absorbance of 0.750 ± 0.025 at 734 nm with ethanol. Then, 2.3 ml of $\text{ABTS}^{+\cdot}$ solution was added 100 μl of CLME solution at different concentrations (0.10 to 0.30 mg/ml). After 30 min, the percentage inhibition of 734 nm was calculated for each concentration relative to a blank absorbance. Solvent blanks were run in each assay. The extent of decolorization is calculated as percentage reduction of absorbance. The scavenging capability of $\text{ABTS}^{+\cdot}$ radical was calculated using the following equation:

$$\text{ABTS}^{+\cdot} \text{ scavenging effect } (\%) = (1 - A_S / A_C) \times 100$$

where A_C is the initial concentration of the $\text{ABTS}^{+\cdot}$ and A_S is absorbance of the remaining concentration of $\text{ABTS}^{+\cdot}$ in the presence of CLME (Gülçin et al., 2010).

Determination of reducing power

The reducing power of CLME solution was determined according to the method of Oyaizu (1986). Different concentrations of CLME solution (0.050, 0.10 and 0.25 mg) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of TCA (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl_3 (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer (Shimadzu, UV/Visible Recording). Higher absorbance of the reaction mixture indicated greater reducing power. Butylated hydroxytoluene and α -tocopherol were used as standards.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenger ability was measured according to a literature procedure with a few modifications (Smirnov and Cumbes, 1989). Hydroxyl radical was generated from Fenton reaction between 1.5 mM FeSO_4 and 6 mM H_2O_2 (1.4:1, v/v) at 37°C for 30 min before the assay, and was detected by their ability to hydroxylate salicylate. The reaction mixture 3.0 ml contained 1.0 ml of 1.5 mM FeSO_4 , 0.7 ml of 6 mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate and varied concentrations of the extracts (0.10, 0.20, and 0.30 mg/ml). After incubation for 1 h at 37°C, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The scavenging activity of hydroxyl radical effect was calculated as follows: $[1 - (A_1 - A_2) / A_0] \times 100$, where A_0 is absorbance of the control (without extract) and A_1 is the absorbance in the presence of the extract, A_2 is the absorbance without sodium salicylate.

Hydrogen peroxide (H_2O_2) scavenging activity assay

Hydrogen peroxide scavenging activity of CLME and standards was assayed by the method of Zhao et al. (2006). H_2O_2 (1.0 ml, 0.1 mM) and 1.0 ml of various concentrations (0.15, 0.30, and 0.45 mg/ml) of the extract were mixed, followed by 100 μl 3% ammonium molybdate, 10 ml H_2SO_4 (2 M) and 7.0 ml KI (1.8 M). The mixed solution was titrated with $\text{Na}_2\text{S}_2\text{O}_3$ (5 mM) until the yellow color

disappeared. The percentage scavenging effect was calculated as:

$$\text{Hydrogen peroxide scavenging rate (\%)} = [(V_C - V_S) / V_C] \times 100,$$

where V_C was the volume of $\text{Na}_2\text{S}_2\text{O}_3$ solution used to titrate the control sample in the presence of hydrogen peroxide (without extract), V_S was the volume of $\text{Na}_2\text{S}_2\text{O}_3$ solution used in the presence of CMLE. Ascorbic acid was used as standard.

Deoxyribose assay

The reaction mixture, containing methanol extract of CLME (0.02 to 0.10 mg/ml) was incubated with deoxyribose (10 mM), H_2O_2 (50 mM), FeCl_3 (10 μM), EDTA (1 mM) and ascorbic acid (10 mM) in potassium phosphate buffer (50 mM, pH 7.4) for 60 min at 37°C (Halliwell et al., 1987). Then, reaction was terminated by adding 1 ml of 10% TBA (1% w/v) and 1 ml of TCA (2% w/v) and then heating the tubes in a boiling water-bath for 15 min. The contents were cooled and the absorbance of the mixture was measured at 532 nm against reagent blank. Decreased absorbance of the reaction mixture indicated decreased oxidation of deoxyribose:

$$\text{Inhibition (\%)} = [(A_C - A_S) / A_C] \times 100,$$

where A_C is the absorbance of the control and A_S is the absorbance in the presence of samples of extracts.

β -Carotene bleaching test

The test was carried out following the spectrophotometric method of Miller (1971), based on the ability of the different extracts to decrease the oxidative bleaching on β -carotene in a β -carotene/linoleic acid emulsion, was used. 2.0 mg sample of crystalline β -carotene was dissolved in 10 ml of chloroform and 1 ml of this solution was added to 20 μl of linoleic acid and 200 μl of Tween-20 in a round-bottom flask. After removing the chloroform in a rotary evaporator under vacuum at 40°C for 5 min, 50 ml distilled water was added to the residue, with vigorous stirring in order to form an emulsion. Five milliliters of this emulsion was added to each tube containing extracts (0.05 mg/ml). A zero reading was taken at 470 nm on the reaction mixture in each tube immediately after addition of the emulsion to the antioxidant solution. A control sample with distilled water instead of extract was also analyzed for antioxidant activity. The tubes were then stoppered and placed in a water bath at 50°C. Subsequent readings were taken at regular intervals until the carotene had been decolorized (about 90 min). All determinations were performed in duplicate. As standard Trolox, α -tocopherol, BHT and ascorbic acid were used. The percentage inhibition was calculated from the data with the slightly modified formula (Kulicic et al., 2004).

$$\text{Inhibition (\%)} = [(A_{S(90)} - A_{C(90)}) / (A_{C(0)} - A_{C(90)})] \times 100$$

where $A_{S(90)}$ is the absorbance of the antioxidant at $t=90$ min, $A_{C(90)}$ is the absorbance of the control at $t=90$ min, and $A_{C(0)}$ is the absorbance of the control at $t=0$ min.

Effect of cherry leaves methanol extract (CLME) on pBR322 super coiled plasmid DNA scission induced by hydroxyl radical

DNA damage protective activities of CLME were checked on pBR322 super coiled plasmid DNA (Vivantis). Plasmid DNA was oxidized with H_2O_2 + UV treatment in the presence of CLME and checked on 1% agarose after modification (Attaguile et al., 2000).

In brief, the experiments were performed in a volume of 10 μl in a microcentrifuge tube containing 200 ng of plasmid DNA in phosphate buffer (7.14 mmol phosphate and 14.29 mmol NaCl), pH 7.4, H_2O_2 was added at a final concentration of 2.5 mmol/L with and without 1 μl of 0.20 and 0.40 mg/ml methanol extract. The reactions were initiated by UV irradiation and continued for 5 min on the surface of a UV transilluminator (8000 $\mu\text{W cm}^{-1}$) at 300 nm at room temperature. After irradiation, the reaction mixture (10 μl) with gel loading dye was placed on 1% agarose gel for electrophoresis. Electrophoresis was performed at 40 V for 3 h in the presence of ethidium bromide (10 mg/ml). Untreated pBR322 super coiled plasmid DNA was used as a control in each run of gel electrophoresis along with partial treatment, that is, only ultraviolet (UV) treatment and only H_2O_2 . Percentage inhibition of the DNA strand scission was calculated as follows:

$$\text{Inhibition (\%)} = 1 - [(S_m + a - S_c) / (S_m - S_c)]$$

where S_m+a is the percentage remaining super coiled after treatment with mix plus agent, S_c is the percentage remaining super coiled in control untreated plasmid, and S_m is the percentage remaining super coiled mix without agent (Fukuhara and Miyata, 1998).

Densitometric analysis of treated and control pBR322 super coiled plasmid DNA

Gel was scanned on the gel documentation system (Gel-Doc-XR, BioRad, Hercules, CA). Bands on the gels were quantified discovery series Quantity One program (version 4.5.2, BioRad).

Statistical analysis

The antioxidant data in the present study were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between the means was determined by the Duncan's multiple range tests at 95% least significant difference ($P < 0.05$).

RESULTS AND DISCUSSION

Polyphenolic contents of the extracts

Phenolic compounds are known as powerful chain breaking antioxidants (Shahidi and Wanasundara, 1992). Phenols are very important plant constituents, because of their scavenging ability due to their hydroxyl groups (Hatano et al., 1989). The phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested up to 1 g daily from a diet rich in fruits and vegetables (Tanaka et al., 1998). The total amount of phenolic content in 1 g methanol extract of cherry leaves 116.4 ± 35.0 mg gallic acid equivalent of phenols was detected.

Antioxidant activity

DPPH radical scavenging activity

The free-radical scavenging activity of methanol extract of CLME was tested by their ability to bleach the stable

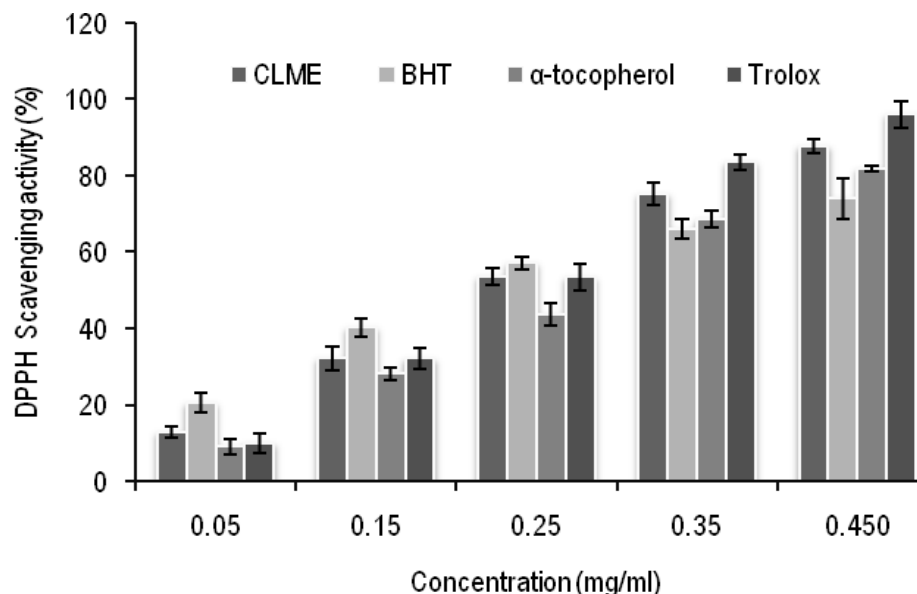


Figure 1. Scavenging effect of CLME on 1,1-diphenyl-2-picrylhydrazyl radicals. Each value is expressed as mean \pm SD (n = 3).

DPPH radical (Saija et al., 1998). This assay provided information on the reactivity of crude extract with stable free radical. The effects of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH gives a strong absorption band at 517 nm in visible spectroscopy (deep violet color). The reduction capability of DPPH radicals was determined by a decrease in absorbance at 517 nm induced by antioxidants. The extract exhibited DPPH free radical scavenging activity in a concentration-dependent manner (Figure 1).

CLME exhibited stronger DPPH scavenging activity. Scavenging activity of CLME and standard compounds followed the order: Trolox > CLME > α -tocopherol > BHT, and were 96.17 ± 3.51 , 87.80 ± 1.73 , 81.92 ± 0.58 and $74.0 \pm 5.29\%$ at 0.45 mg/ml concentration, respectively.

ABTS radical-scavenging activity assay

Another effective method to measure radical scavenging activity is the ABTS radical cation decolorization assay, which showed similar results to those obtained in the DPPH reaction. The ABTS radical scavenging by CLME and standard antioxidant decreased in the following order: Trolox (89.98%) and CLME (81.95%) at a concentration of 0.30 mg/ml test sample. Also, the percentages of ABTS cation radical scavenging activity of different concentrations (0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 mg/ml) of CLME were found to be 25.37, 41.72,

51.27, 57.96, 77.81 and 81.95%, respectively. These results show that CLME has effective ABTS cation radical scavenging activity at higher concentration (Figure 2). However, these values are lower than those of standard antioxidant.

Reducing power

The reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and non-reactive species (Gülçin et al., 2007). The reducing power of CLME was investigated by FRAP. Antioxidant compounds reduce Fe^{3+} -ferricyanide complexes to the ferrous (Fe^{2+}) form. The Prussian blue colored complex is formed by adding FeCl_3 to the ferrous (Fe^{2+}) form. Therefore, the amount of reduction can be determined by measuring the formation of Perl's Prussian blue at 700 nm (Chung et al., 2002). In this assay, the yellow color of the test solution changes to green or blue depending on the reducing power of the antioxidant. A higher absorbance indicates higher ferric reducing power. As shown in Figure 3, the ferric reducing power of CLME increased with increasing concentration, similar to the standard antioxidants. However, these differences were found as insignificant ($p > 0.05$). The reducing power of the CLME and standard antioxidants decreased in the following order: BHT > Trolox > α -tocopherol > CLME with 0.25 mg/ml test sample. CLME had a similar ferric reducing power to standard antioxidants (BHT, α -tocopherol and Trolox). Fe^{3+} reduction is often used as an

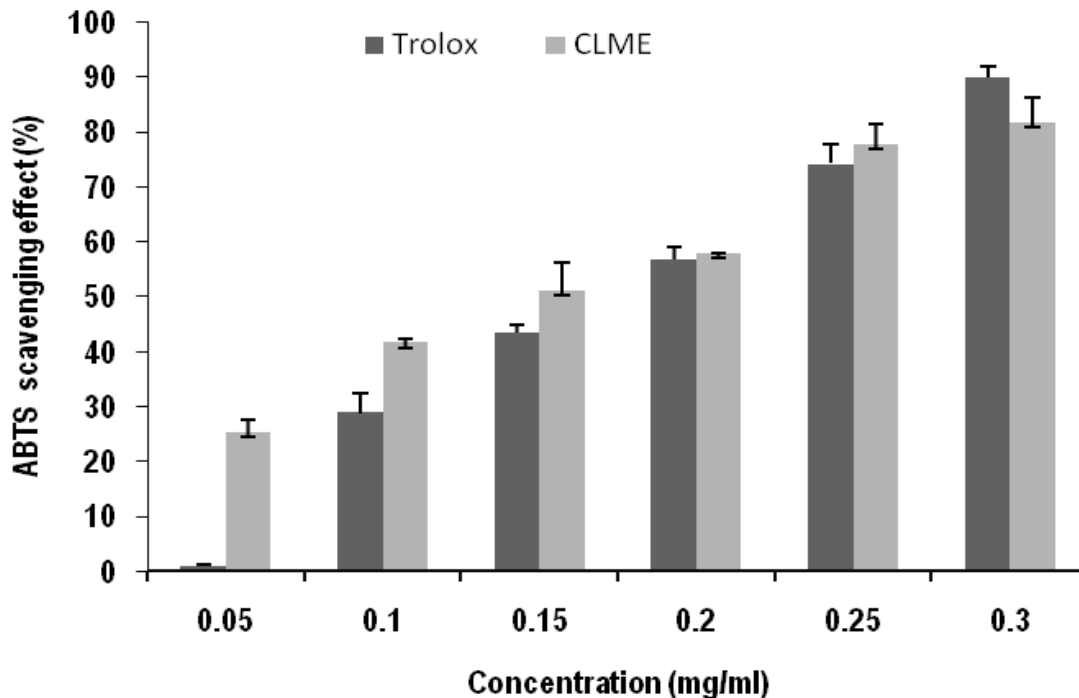


Figure 2. ABTS^{•+} radical scavenging activity of CLME.

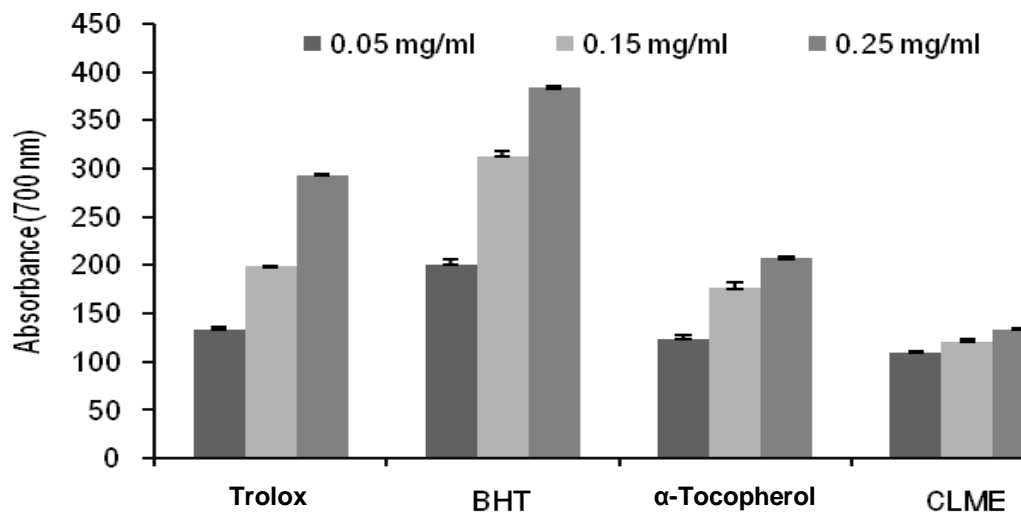


Figure 3. Reducing power of CLME.

indicator of electron-donating activity, which is an important mechanism of phenolic antioxidants (Dorman et al., 2003).

Hydroxyl radical scavenging activity

The hydroxyl radical is the most reactive of the ROS, and it induces severe damage in adjacent biomolecules

(Gutteridge, 1984). The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins (Spencer et al., 1994). The $\cdot\text{OH}$ scavenging activity of mushroom extracts was assessed by its ability to compete with salicylic acid for $\cdot\text{OH}$ radicals in the $\cdot\text{OH}$ generating/detecting system. In the present study, the hydroxyl radical-scavenging effect of the CLME, in a concentration of 0.1 mg/ml, was found to be 45.33% and in a concentration of 0.2 mg/ml, was found to be 43.99% (Figure 4).

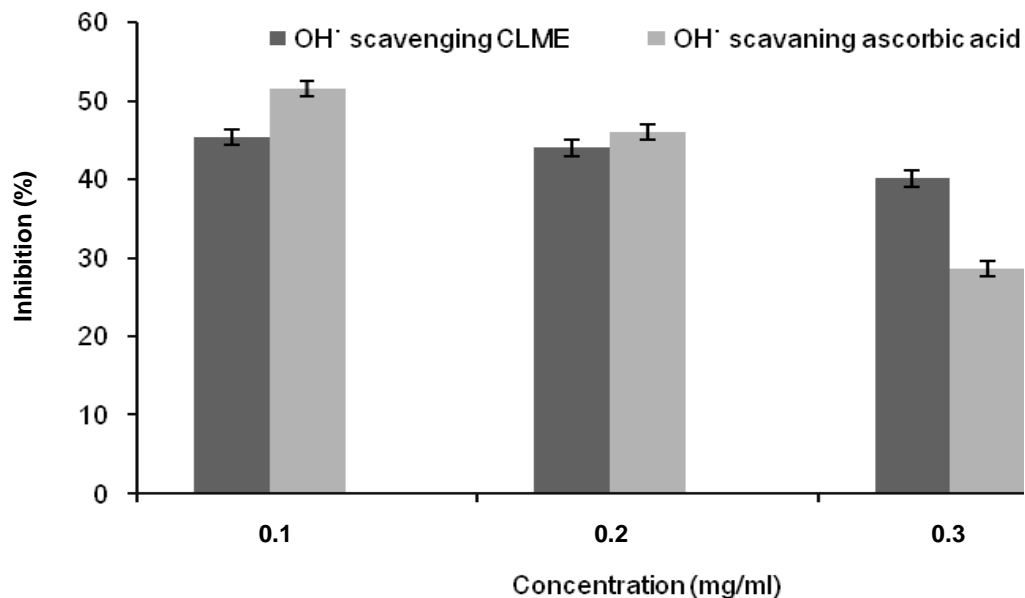


Figure 4. Hydroxyl radical scavenging activity of CLME.

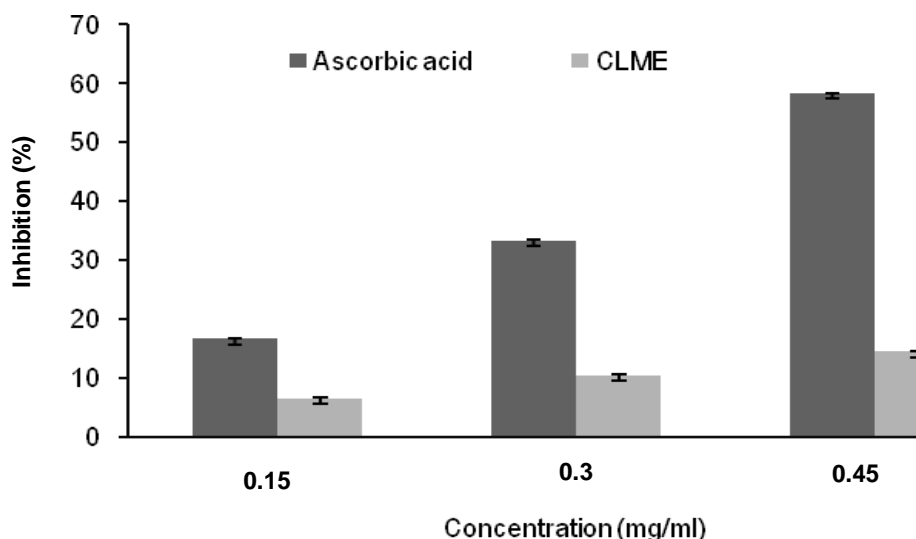


Figure 5. Hydrogen peroxide radical scavenging of CLME compared to that of ascorbic acid (Vitamin C).

Each value is expressed as mean \pm standard deviation (n=3).

Hydrogen peroxide radical scavenging activity

The free radical scavenging activity of CLME was evaluated by hydrogen peroxide (H_2O_2) scavenging method. From the results, CLME showed concentration dependent activity and the H_2O_2 scavenging effect was 14.47% at a concentration of 0.45 mg/ml. This was comparable to the scavenging effect of ascorbic acid (58.33%) (Figure 5).

Deoxyribose assay

The deoxyribose method is a simple assay to determine the rate constants for reactions of hydroxyl radicals. When the mixture of $FeCl_3$ -EDTA, H_2O_2 and ascorbate were incubated with deoxyribose, in phosphate buffer (pH7.4), the generated hydroxyl radicals attack the deoxyribose and result in a series of reactions that cause the formation of malondialdehyde (MDA). Any hydroxyl

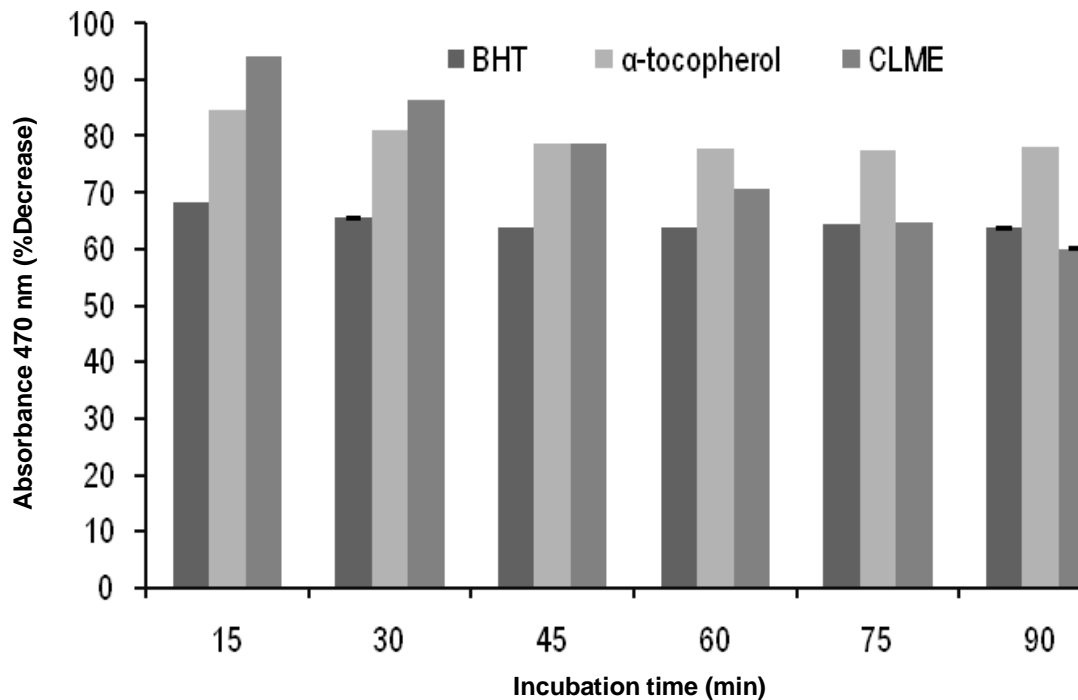


Figure 6. Relative changes in absorbance of beta carotene emulsions containing BHT, α -tocopherol and CLME.

radical scavenger added to reaction would compete with deoxyribose for the availability of hydroxyl radicals, thus reducing the amount of MDA formation (Wang et al., 2003). It has been found that the CLME showed concentration-independent scavenging activity on hydroxyl radicals (Figure 7). CLME exhibited 97% scavenging capacity at the 0.02 mg/ml concentration.

Antioxidant assay using the β -carotene bleaching method

The mechanism of beta-carotene bleaching is a free radical-mediated phenomenon resulting from the hydroperoxides formed from linoleic acid by air oxidation. The antioxidant activity of carotenoids is based on the radical adducts of carotenoids with free radicals formed from linoleic acid. The linoleic acid free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated beta-carotene molecules. As beta-carotene molecules lose their double bonds by oxidation in the absence of an antioxidant, the compound loses its chromophore and characteristic orange color, which the anti-bleaching activity of sample of β -carotene was studied by monitoring the color intensity of emulsion at 470 nm for every 15 min for 90 min. The concentration taken was 0.05 mg/ml for the sample as well as standard (BHT and α -tocopherol). The initial concentration was considered to

be 100%. As shown in Figure 6, in the first 15 min, the CLME showed 93.96% bleaching as compared to 68.27 and 84.50% to that of standard (BHT and α -tocopherol). In 60 min of incubation, percentage decrease was found to be 70.75 and 63.84%; 77.81% for CLME and standard (BHT; α -tocopherol), respectively. During the 90 min, it came to 60.06 and 63.69%; 78.17% for CLME and standard (BHT; α -tocopherol), respectively.

DNA damage protective activity of CLME

ROS-induced DNA damage can be described both chemically and structurally and shows a characteristic pattern of modification. It is well known that in various cancer tissues free radical-mediated DNA damage was found (Valko et al., 2001). The majority of these changes can be reproduced by ROS experimentally including the following: modification of all bases, production of base-free sites, deletions, frame shifts, strand breaks, DNA-protein cross-links, and chromosomal rearrangement. An important reaction involved in DNA damage involves generation of hydroxyl radical, e.g., through Fenton chemistry (Brezova et al., 2003). Hydroxyl radical is known to react with all components of the DNA molecule: the purine and pyrimidine bases as well as the deoxyribose backbone (Valko et al., 2004). When DNA was exposed to H_2O_2 and irradiated with UV light, H_2O_2 will be generated to hydroxyl radicals, then the super

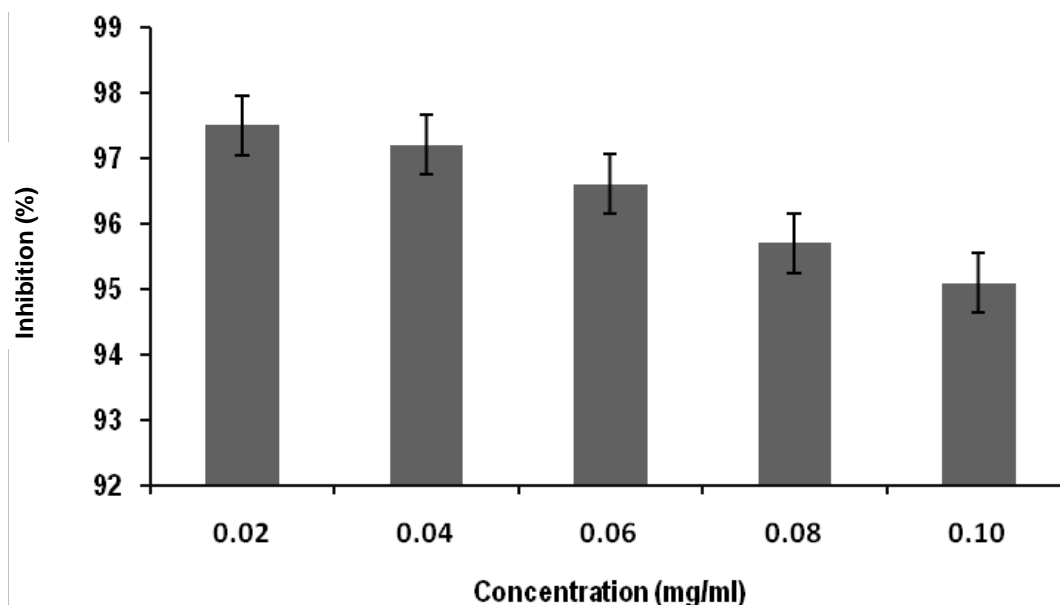


Figure 7. Effect of methanol extracts of CLME on deoxyribose degradation assay. Each value is expressed as mean of 3 replicates \pm standard deviation.

coiled form of DNA would cleave.

DNA damage protective activity of CLME was investigated with pBR322 super coiled plasmid DNA (Vivantis). Figure 8A shows the quantified band intensity for the super coiled-DNA (form I), circular relaxed -DNA (form II) and linear-DNA (form III). Figure 8B shows the electrophoretic pattern of DNA after UV-photolysis of H_2O_2 (2.5 mM) in the absence and presence of CLME (0.20 and 0.40 mg/ml). DNA derived from pBR322 super coiled plasmid DNA showed two bands on agarose gel electrophoresis (lane 1), the faster moving band corresponded to the native form of super coiled circular DNA and the slower moving band was the circular relaxed DNA form. The UV irradiation of DNA in the presence of H_2O_2 (lane 3) resulted in the cleavage of super coiled DNA to linear DNA form, indicating that OH radical generated from UV photolysis of H_2O_2 produced DNA strand scission. The addition of extract (lanes 5 to 7) to the reaction mixture suppressed the formation of linear DNA and induced a partial recovery of super coiled DNA.

In fact, the intensity of super coiled DNA bands scanned from the agarose gel electrophoretic patterns was 95.7 and 96.2% for plasmid DNA treated with H_2O_2 in the presence of 0.20 and 0.40 mg/ml extract, respectively, as compared with the untreated plasmid DNA.

Thus, the identification of natural products able to provide protection against UV radiation-induced inflammatory responses and the generation of oxidative stress may have important human health implications. The DNA cleavage analysis demonstrated the strong antioxidant properties of CLME. In fact, this extract suppressed the formation of linear DNA, generated by exposure of plasmid

DNA to OH radical generated by H_2O_2 UV-photolysis, and induced a partial recovery of super coiled DNA. DNA damage protecting activity of CLME is corresponding to its antioxidant potential. It is known that metal-induced generation of oxygen radicals results in the attack of not only DNA in cell nucleus, but also other cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation (Esterbauer et al., 1991; Marnett, 1999). The initial products of unsaturated fatty acid oxidation are short-lived lipid hydroperoxides. When they react with metals they produce a number of products (e.g., aldehydes and epoxides) which are themselves reactive. MDA is one of the major aldehyde products of lipid peroxidation. It is mutagenic in mammalian cells and carcinogenic in rats (Valko et al., 2004).

Conclusion

This study is the first to evaluate the antioxidant activity of CLME in a comprehensive manner employing a variety of *in vitro* methods. It was reported in this study that an extract from CLME was active in scavenging OH radicals in a deoxyribose assay, as well as quenching the stable free radical DPPH. The antioxidant potential of CLME was also further demonstrated through its reducing activity and total polyphenol content. The results of the present study would certainly help to ascertain the potency of the crude extract of CLME as a potential source of natural antioxidants. This work has gathered experimental evidence on the commonly used CLME as natural antioxidant for its capacity to protect organisms

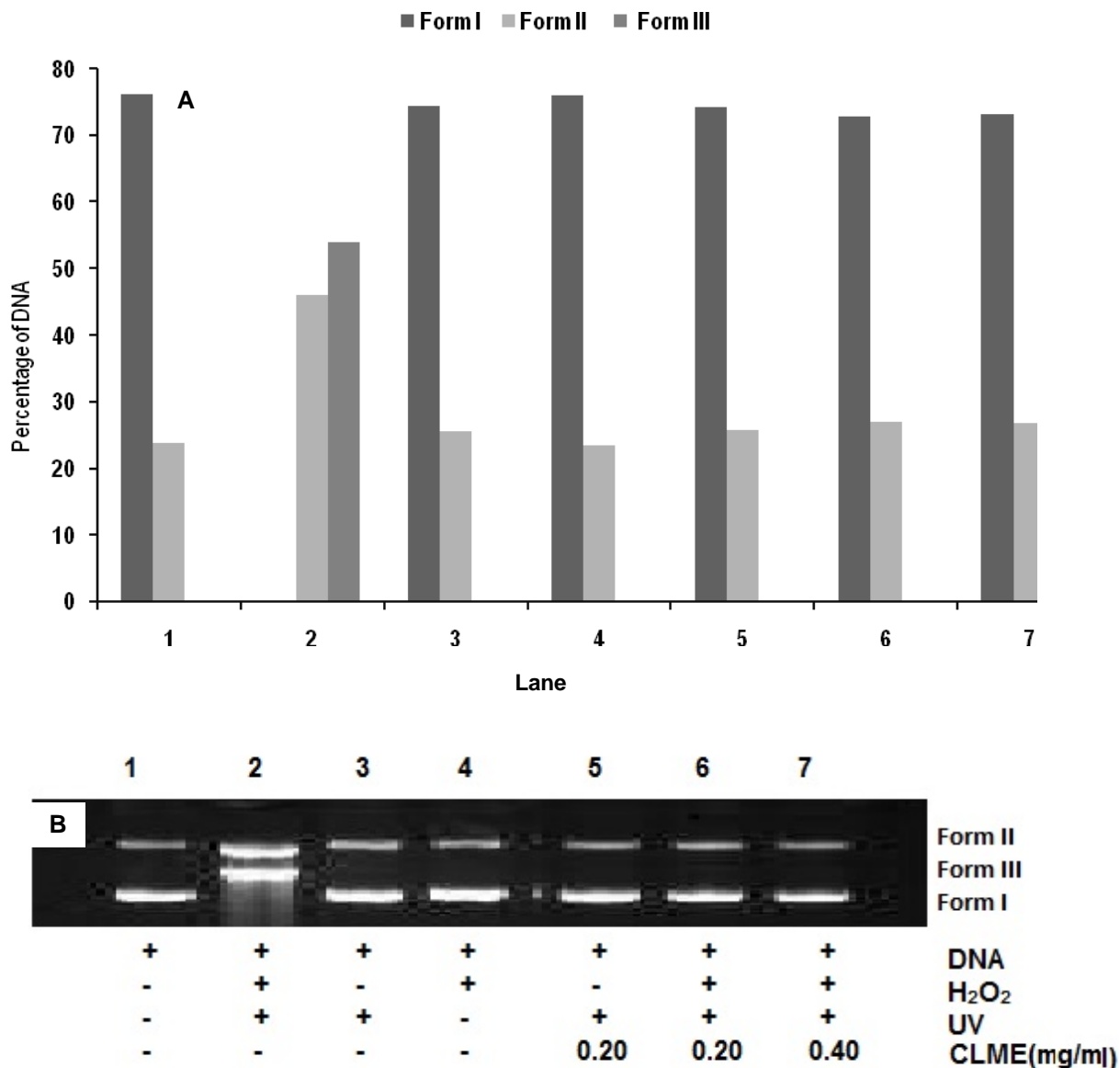


Figure 8. (A) The quantified band intensity for the sc-DNA (form I), oc-DNA (form II) and I-DNA (form III) with Quantity One 4.5.2. version software. (B) Electrophoretic pattern of pBR322 super coiled plasmid DNA after UV-photolysis of H₂O₂ in the presence or absence of CLME. Reaction vials contained 200 ng of super coiled DNA (31.53 nM) in distilled water, pH 7. Electrophoresis was performed using 1% agarose at 40 V for 3 h in the presence of ethidium bromide (10 mg/ml). Electrophoresis running buffer: TAE (40 mM Tris acetate, 1 mM EDTA, pH 8.2). Lane 1, control DNA; Lane 2, DNA + H₂O₂ (2.5 mM)+UV; Lane 3, DNA + UV; Lane 4, DNA + H₂O₂ (2.5 mM); Lane 5, DNA + CLME (0.20 mg/ml) + UV; Lane 6, DNA + CLME (0.20 mg/ml) + H₂O₂ (2.5 mM) + UV; Lane 7, DNA + CLME (0.40 mg/ml) + H₂O₂.

and cells from oxidative DNA damage associated with aging, cancer and degenerative diseases. This profound protective effect of CLME against oxidative DNA damage, free radicals scavenging, and inhibition of lipid peroxidation may explain its extensive use in daily life and possible health benefits.

Thus, CLME may serve as an ideal candidate for a cost-effective, readily exploitable natural polyphenolic phytochemical. However, further research is needed to

identify individual components forming the antioxidative system and develop their application for food and pharmaceutical industries.

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Conflict of Interest

Authors declare no conflict of interest.

REFERENCES

- Alasalvar C, Al-Farsi M, Shahidi F (2005). Compositional characteristics and antioxidant components of cherry laurel varieties and pekmez. *J. Food Sci.* 70(1):47–52.
- Reiger M (2003). Cherry – *Prunus avium*, *Prunus cerasus*. Available at: <http://www.fruit-crops.com/cherry-prunus-avium-cerasus>
- Attaguile G, Russo A, Campisi F, Savoca F, Acquaviva R, Ragusa N, Vanella A (2000). Antioxidant activity and protective effect on DNA cleavage of extracts from *Cistus incanus* L. and *Cistus monspeliensis* L. *Cell. Biol. Toxicol.* 16:83–90.
- Barlow SM (1990). Toxicology aspects of antioxidants used as food additives. In *Food antioxidants*; Hudson BJJ; Eds.; Elsevier: New York p. 253.
- Bingham M, Gibson G, Gottstein N, Pascual-Teresa SD, Minihane AM, Rimbach G (2003). Gut metabolism and cardio protective effects of dietary isoflavones. *Curr. Topic Nutraceut. Res.* 1:31–48.
- Saija A, Tomaino A, Lo Cascio R, Rapisarda P, Dederen JC (1998). *In vitro* antioxidant activity and *in vivo* photoprotective effect of red orange extract. *Int. J. Cosmet. Sci.* 20:331–342.
- Brezova V, Valko M, Breza M, Morris H, Telsler J, Dvoranova D, Kaiserova K, Varecka L, Mazur M, Leibfritz D (2003). Role of radicals and singlet oxygen in photoactivated DNA cleavage by the anticancer drug camptothecin: An electron paramagnetic resonance study. *J. Phys. Chem. B.* 107:2415–2425.
- Chung YC, Chang CT, Chao WW, Lin CF, Chou ST (2002). Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR- NK1. *J. Agric. Food Chem.* 50:2454–2458.
- Dorman HJD, Peltoketo A, Hiltunen R, Tikkanen MJ (2003). Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. *Food Chem.* 83:255–262.
- Elahi MM, Kong YX, Matata BM (2009). Oxidative stress as a mediator of cardiovascular disease. *Oxid. Med. Cell Longev.* 2(5):259–269.
- Esterbauer H, Schaur RJ, Zollner H (1991). Chemistry and biochemistry of 4-hydroxydesoxy-nonenal, malonaldehyde and related aldehydes. *Free Radic. Biol. Med.* 11:81–128.
- FAOSTAT (2011). Trade, major commodity exporters. Available at: <http://faostat.fao.org/site/342/default.aspx>
- Fukuhara K, Miyata N (1998). Resveratrol as a new type of DNA cleaving agent. *Bioorg. Med. Chem. Lett.* 8:3187–3192.
- Gülçin İ, Elmastas M, Aboul-Enein HY (2007). Determination of antioxidant and radical scavenging activity of basil (*Ocimum basilicum*) assayed by different methodologies. *Phytother. Res.* 21:354–361.
- Gülçin İ, Huyut Z, Elmastaş M, Aboul-Enein HY (2010). Radical scavenging and antioxidant activity of tannic acid. *Arab. J. Chem.* 3:43–53
- Gutteridge MC (1984). Reactivity of hydroxyl and hydroxyl-like radicals discriminated by release of thiobarbituric acid reactive material from deoxy sugars, nucleosides and benzoate. *Biochem. J.* 224:761–767.
- Halliwell B, Gutteridge JMC, Auroma OI (1987). The deoxyribose method: A simple “test tube” assay for determination of rate constants for reaction of hydroxyl radicals. *Anal. Biochem.* 165:215–219.
- Hatano T, Edamatsu R, Mori A, Fujita Y, Yasuhara T, Okuda T (1989). Effect of interaction of Tannins With co-existing substances. *Chem. Pharmacol. Bull.* 37:2016–2021.
- Islam A (2002). “Kiraz” cherry laurel (*Prunus laurocerasus*). *New Zealand J. Crop Hortic. Sci.* 30:301–302.
- Jung HA, Kim AR, Chung HY, Choi JS (2002). *In vitro* antioxidant activity of some selected *Prunus* species in Korea. *Arch. Pharm. Res.* 25:865–72.
- Jung HA, Chung HY, Kang SS, Hyun SK, Kang HS, Choi JS (2005). A phenolic Glucoside isolated from *Prunus serrulata* var. *spontanea* and its peroxynitrite scavenging activity. *Arch. Pharm. Res.* 28:1127–30.
- Kolayli S, Küçük M, Duran C, Candan F, Dinçer B (2003). Chemical and antioxidant properties of *Laurocerasus officinalis* Roem. (Cherry laurel) fruit grown in the Black Sea region. *J. Agric. Food Chem.* 51:7489–7494.
- Kulisic T, Radonic A, Katalinic V, Milos M (2004). Analytical, Nutritional and Clinical Methods; Use of different methods for testing antioxidant activity of oregano essential oil. *Food Chem.* 85:633–640
- Liyana-Pathirana CM, Shahidi F, Alasalvar C (2006) Antioxidant activity of cherry laurel fruit (*Laurocerasus officinalis* Roem.) and its concentrated juice. *Food Chem.* 99:121–128.
- Marnett LJ (1999). Lipid peroxidation-DNA damage by malondialdehyde. *Mutat. Res. Fundam. Mol. Mech. Mugag.* 424:83–95.
- Miller HE (1971). A simplified method for the evaluation of antioxidants. *J. Am. Oil Chem. Soc.* 48:91–97.
- Oyaizu M (1986). Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. *J. Nutr.* 44:307–315.
- Praticò D (2008). Evidence of oxidative stress in Alzheimer’s disease brain and antioxidant therapy: lights And shadows. *Ann. N. Y. Acad. Sci.* 1147:70–78.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant Activity applying an improved ABTS radical cation decolorization assay. *Free Rad. Biol. Med.* 26:1231–1237
- Shahidi F, Wanasundara PD (1992). Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* 32:67–103.
- Silva BM, Andrade PB, Valentão P, Ferreres F, Seabra RM, Ferreira MA (2004). Quince (*Cydonia oblonga* Miller) fruit (pulp, peel, and seed) and jam: Antioxidant activity. *J. Agric. Food Chem.* 52:4705–4712.
- Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Met. Enzymol.* 299:152–78
- Smirnoff N, Cumbes QJ (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry* 28: 1057–1060.
- Spencer JPE, Jenner A, Aruoma OI (1994). Intense oxidative DNA damage promoted by L-DOPA and its metabolites, implications for neurodegenerative disease. *FEBS Lett.* 353:246–250.
- Taguchi N, Uemura N, Goto Y, Sakura M, Hara K, Niwa M, Iida M, Takeshi Yanagishita T, Watanabe D, Kato M (2011). Antioxidative effects of cherry leaves extract on tert-butylhydroperoxide mediated cytotoxicity through regulation of thioredoxin-2 protein expression levels. *J. Toxic. Environ. Health Part A.* 74:1240–1247.
- Tanaka M, Kuei CW, Nagashima Y (1998). Application of antioxidative Maillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishi* 47:1409–1414
- Uttara B, Singh AV, Zamboni P, Mahajan RT (2009). Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. *Curr. Neuropharmacol.* 7(1):65–74.
- Valko M, Mario I, Mazur M, Rhodes CJ, Telsler J (2004). Role of oxygen radicals in DNA damage and cancer incidence. *Mol. Cell. Biochem.* 266:37–56.
- Valko M, Morris H, Mazur M, Raptap P, Bilton RF (2001). Oxygen free radical generating mechanisms in the colon: Do the semiquinones of vitamin K play a role in the aetiology of colon cancer. *Biochim. Biophys. Acta* 1527:161–166.
- Wang L, Yen JH, Liang HI (2003). Antioxidant effect of methanol extracts from *Lotus plumule* and blossom (*Nelumbo nufera* Gertn.). *J. Food Drug Anal.* 11:60–66.
- Waris G, Ahsan H (2006). Reactive oxygen species: role in the development of cancer and various chronic conditions. *J. Carcinog.* 5(1):14–21.
- Wright Jr. E, Scism-Bacon JL, Glass LC (2006). Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. *Int. J. Clin.*

- Pract. 60(3):308-314.
- Yen GC, Chien H (2000). Effects of Alkaline and Heat Treatment on Antioxidative Activity and Total Phenolics of Extracts from Hsian-Tsao (*Mesona Procumbens* Hemsl.), Food Res. Int. 33:487-492.
- Zhao GR, Xiang ZJ, Ye TX, Yuan YJ (2006). Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*. Food Chem. 99:767-774.
- Zhou C, Huang Y, Przedborski S (2008). Oxidative stress in Parkinson's disease: a mechanism of Pathogenic and therapeutic significance. Ann. N. Y. Acad. Sci. 1147:93-104.
- Zohary D, Hopf M (2000). Domestication of Plants in the Old World, 3rd Edition. Oxford University Press, New York. P 182.



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