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Full Length Research Paper

The effect of blending of extracts of Sudanese *Adansonia digitata* and *Tamarindus indica* on their antioxidant, anti-inflammatory and antimicrobial activities

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***Adansonia digitata* (Bombacaceae) *and Tamarindus indica* (Fabaceae) are the most popular herbal products in Sudan; they are used as food ingredients and in traditional treatments of various diseases. The aim of this study is to investigate phytochemical contents, antioxidant, anti-inflammatory and antimicrobial activities of plants fruit, leaf and bark ethanolic extracts. The phytochemical screening of different extracts revealed the presence of alkaloids, flavonoids, sterols/triterpens, tannins, saponins, coumarins, glycosides, reducing sugar, lignin and carbohydrates. The results indicate that all the extracts have reducing power DPPH radical scavenging abilities. The highest antioxidant activity showed in *A. digitata* parts. The fruit extracts of both plants showed the highest antioxidant activity (84.07 and 83.98% for *A. digitata* and *T. indica,* respectively). The *in vitro* anti-inflammatory effects have been studied by human albumin denaturation, and both plant extracts showed remarkable activity. Leaf extracts showed highest anti-inflammatory activity (76.714, 62 and 82.71% for *A. digitata*, *T. indica* and mixture of both respectively). The results of antimicrobial activity showed the effectiveness of mixture extracts against tested standard pathogens. Fruit mixtures showed the highest activity against *B. subtilis* (19 mm), *S. aureus* (35 mm) and *S. typhi* (21 mm), while the mixture of bark extracts showed the highest activity against *E. coli* (19 mm). It is noteworthy that higher antioxidant, anti-inflammatory and antimicrobial activities have been observed by blends in the ratio 1:1 of fruit, leaf and bark extracts of both plants.**

Key words: *Adansonia digitata*, *Tamarindus indica*, antioxidant, anti-inflammatory, antimicrobial, combination.

INTRODUCTION

Sudan traditional medicine is characterized by a unique combination of Islamic, Arabic, and African cultures. In

poor communities, traditional medicine has remained as the most reasonable source of treatment of several diseases and microbial infections. Although the traditional medicine is accepted in Sudan, to date there is no updated review available which focuses on most effective and frequently used Sudanese medicinal plants (Karar and Kuhnert, 2017) .

Adansonia digitata (Bombacaceae) known as Baobab is an important plant used in Sudanese traditional medicine; it is widespread throughout the hot, drier regions of tropical Africa. It extends from northern Transvaal and Namibia to Ethiopia and Sudan. In Sudan, the baobab is most frequently found on sandy soils and by seasonal streams Khors in short grass savannas. It forms belts in Central Sudan, Kordofan, Darfur and Blue Nile (Dabora, 2016). The Baobab fruit pulp is an important foodstuff used as a drink, a sauce for food and as a fermenting agent in local brewing (Gebauer et al., 2002). Different parts of the plant are used to treat many diseases. The alkaloid 'adansonin' in the bark is thought to be the active principle for treatment of malaria and other fevers (De Caluwé et al., 2010). The plant is: Anti-oxidizing agent including; polyphenolic compounds, vitamins E and C, cardiovascular diseases, cancer and aging related disorders (De Caluwé et al., 2010); antiviral activity against Herpes simplex, Sindbis and Polio (Anani et al., 2000); anti-inflammatory and antipyretic activity (Kaboré et al., 2011); anti-microbial activity and anti-trypanosoma activity (Varudharaj et al., 2015). *Tamarindus indica* (Fabaceae) grows wild in Africa in locales as diverse as Sudan, Cameroon, Nigeria, and Tanzania (Havinga et al., 2010). Tamarind fruit pulp is used for seasoning as a food component to flavour confections, curries and sauces, and is a main component in juices and certain beverages. Tamarind fruit pulp is eaten fresh and often made into a juice, infusion or brine, and can also be processed into jam and sweets (Hassan, 2014). The plant is widely used in African traditional medicine for treatment of many diseases such as fever, dysentery, jaundice, gonococci and gastrointestinal disorders (Lawal et al., 2010). Phytochemical investigation carried out revealed the presence of many active constituents, such as phenolic compounds, cardiac glycosides , L-(-)mallic acid, tartaric acid, the mucilage and pectin, arabinose, xylose, galactose, glucose, and uronic acid. The ethanolic extracts showed the presence of fatty acids and various essential elements like arsenic, calcium, cadmium, copper, iron, sodium, manganese, magnesium, potassium, phosphorus, lead, and zinc (Bhadoriya et al., 2011). Many studies have been performed on skin, eye and respiratory tract irritation mediated by complex mixtures, but only few studies allows a quantitative evaluation of the modulating effects of the combination of single chemicals. Further studies on the modulating effects of the combination of chemicals concerning skin, eye and respiratory tract irritation will be required in order to evaluate the possibilities for synergistic or antagonistic effects being mediated by mixtures of chemicals (Doty et al., 2004). The present study reports the effect of blending equal amounts of *A. digitata* (Bombacaceae) and *T. indica* (Fabaceae) fruit, leaf and bark 96% ethanolic extract on their antioxidant, anti-inflammatory and antimicrobial activities.

MATERIALS AND METHODS

Preparation of plants extracts

The fruit, leaves and bark of *A. digitata* and *T. indica* were collected from Abu karshola,West Kordofan State, Sudan, in December, 2017. The specimens were deposited in the herbarium of medicinal and aromatic plants institute, Khartoum, Sudan. The fresh samples were cleaned, air dried and ground to powder using a pestle and mortar. Fifty grams of each powdered sample was extracted with 96% ethanol at room temperature for 72 h, filtered through Whatman number 4 filter paper and concentrated in a rotatory evaporator under reduced pressure.

Phytochemical analysis

Qualitative preliminary phytochemical analysis was performed with different chemical reagents to detect the nature of phytoconstituents and their presence in the samples. The presence of sterols/terpenes, flavonoids, tannins, alkaloids, lignins, saponins and coumarins was evaluated by standard qualitative methods (Hameed, 2012).

Antioxidant activity

The DPPH radical scavenging was determined with some modification (Shimada et al., 1992). In 96-wells plate, the test samples were allowed to react with 2,2-Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour. The concentration of DPPH was kept as 300 µl. The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using Shimadzu UV spectrophotometer double beam. Ascorbic acid was used as standard. The ability to scavenge of the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging activity } (\%) = \frac{(\text{A0} - \text{A1})}{\text{A0}} \times 100$$

Where A0 is the absorbance of the control and A1 is the absorbance of the sample.

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Table 1. Yield percentage of different parts extracts of *A.digitata* and *T.indica.*

Sample	Yield (%)	
	A. digitata	***T.indica***
Fruit	4.64	22.84
Leave	14.7	14.5
Bark	1.9	5.08

Anti-inflammatory activity

Inhibition of albumin denaturation

Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi with slight modification: 500 µL of 1% bovine serum albumin was added to 100 µL of plant extract with different concentrations. This mixture was kept at room temperature for 10 min, followed by heating at 51°C for 20 min. The absorbance was recorded at 660 nm. (Chandra et al., 2012). Percent inhibition for protein denaturation was calculated using:

$$\% \text{ Inhibition} = \frac{(\text{A0} - \text{A1})}{\text{A0}} \times 100$$

Where A0 is the absorbance of the control and A1 is the absorbance of the sample.

Antimicrobial activity

Test microorganisms

Bacteria organisms used were *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. The fungal organisms used were *Candida albicans* and *Aspergillus niger*. Standard strains of microorganism used in this study were obtained from medicinal and aromatic plants research institute.

Antibacterial assay

The disc-diffusion assay with some modifications was employed to investigate the inhibition of bacterial growth by plants extract (Kil et al., 2009). Extract solution (20 mg/ml) was prepared by diluting with dimethyl sulfoxide (DMSO) 30%. Base plates were prepared by pouring 10 ml Mueller-Hinton (MH) agar into sterile Petri dishes. About 0.1 ml of the standardized bacterial stock suspension 10^8 to 10^9 C.F.U/ ml were streaked on Mueller Hinton agar medium plates using sterile cotton swab. Sterilized filter paper disc (6 mm diameter) were soaked in the prepared extracts, and then were placed on the surface of the test bacteria plates. The plates were incubated for 24 h and the diameters of the inhibition zones were measured.

Antifungal assay

The same method described for bacteria was employed to assess antifungal activity, Sabouraud Dextrose Agar was used. The inoculated medium was incubated at 25°C for two days for the *C. albicans* and three days for *A. niger*.

RESULTS AND DISCUSSION

Extraction of the plants

Among the extracts obtained using 96% ethanol, *T. indica* fruits gave the highest yield of 22.84% as shown in Table 1, leaf extracts of both plants gave the same yield of 14%, while the bark extract of *A. digitata* gave the lowest yield of 1.9%.

Phytochemical screening of *T. indica* and *A. digitata*

The extracts of *T. indica* contained some secondary metabolites; most of them present in leaves and bark including alkaloids, flavonoids, glycosides, terpenoids, coumarins, tannins, saponins, reducing sugars and carbohydrates. Antibacterial activity of Tamarind leaf extract was attributed to the presence of flavonoids, alkaloids, tannins, cyanogenic glycosides and anthraquinones. It is known that these phytochemicals and some other aromatic secondary metabolites may serve as natural agents that protect plants against microbial pathogens and insect predators. Phytochemicals may act like antioxidants to facilitate, protect and regenerate essential nutrients and/or work to deactivate cancer-causing substances (Gomathi et al., 2017). The results of the phytochemical screening showed that *A. digitata* fruit, leaf and bark extracts were rich in alkaloids, flavonoides, sterols, triterpines, tannins, saponins, coumarins, glycosides, reducing sugar and carbohydrates in all parts, but anthraquinones were not detected (Table 2). Many of these are known to provide protection against insects' attacks and plant diseases. The results obtained seems to justify the use of leaf of *A. digitata* in African dishes as it contained appreciable amount of some important compounds such as phenols, saponins, flavonoids, alkaloids. It is also possible that these plant species could have allelopathy effect on other organisms in their ecotype since these bioactive substances are responsible for such actions. Cardiac glycoside detected in this plant indicated that the plant could be a good source for birds and insect repellants (Lock et al., 2016).

Table 2. Phytochemical screening of *T. indica* and *A. digitata* parts extracts.

Test	Specific test	Fruit		Leave		Bark	
		T. indica	***A. digitata***	***T. indica***	***A. digitata***	***T. indica***	***A. digitata***
Alkaloids	Wagner's	-ve	-ve	-ve	+ve	+ve	-ve
	Mayer's	-ve	-ve	+ve	-ve	-ve	-ve
	Dragendroff's	-ve	-ve	+ve	-ve	+ve	-ve
Flavonoids	$FeCl_3$	-ve	+ve	-ve	-ve	+ve	+ve
	Lead acetate	+ve	-ve	-ve	+ve	+ve	+ve
Sterols	Salkowski	+ve	+ve	+ve	+-ve	+ve	+ve
	Lebermann	-ve	+ve	+ve	+ve	-ve	+ve
Triterpines	Salkowski	-ve	-ve	-ve	-ve	-ve	-ve
	Leberman	-ve	+ve	-ve	-ve	+ve	+ve
Tannins	Gelatin	+ve	-ve	+ve	+ve	+ve	+ve
	lead acetate	+ve	-ve	-ve	-ve	-ve	-ve
Saponins	Foam test	-ve	+ve	+ve	-ve	-ve	+ve
Coumarin	UV lamp	-ve	+ve	-ve	-ve	+ve	-ve
Glycosides	Keller kiliani	+ve	+ve	+ve	+ve	+ve	+ve
	Kedd's	-ve	-ve	-ve	-ve	+ve	+ve
Reducing sugar	Fehlings	+ve	+ve	-ve	-ve	+ve	-ve
Lignin	Labat test	-ve	-ve	+ve	-ve	-ve	-ve
Carbohydrate	Molich	+ve	+ve	+ve	+ve	+ve	+ve

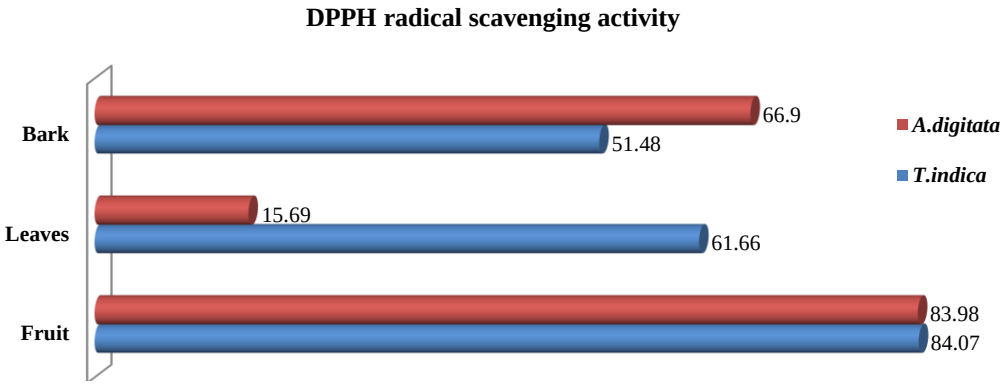

Figure 1. Antioxidant activity of *T. indica* and *A. digitata.*

Antioxidant activity

DPPH radical scavenging activity

The *in vitro* antioxidant activity of fruit, leaf and bark ethanolic extracts of *T. indica* and *A. digitata* was evaluated using DPPH assay. Results are shown in Figure 1. The highest result of antioxidant activity by DPPH scavenging assay in fruit extract in Tamarind (84.07%) followed by Baobab (83.98%). The result is very high compared with ascorbic acid (93.5%) as antioxidant standard. The leaves of Tamarind showed

Table 3. Inhibition of albumin denaturation**.**

Sample	*T. indica* (% inhibition)	*A. digitata* (% inhibition)	Mixture (% inhibition)
Fruit	55.85	50	73.42
Leave	76.714	62	82.71
Bark	57.642	54.92	59.14

Table 4. Antimicrobial activity of plant extracts against bacteria pathogens.

Part of plant	Sample	*Bacteria strain* (M.D.I.Z)*			
		B. subtilis	*S. aureus*	*E. coli*	*S. typhi*
Fruit	*A. digitata*	12 ±0.00	14±1.41	11±1.41	13±0.70
	T. indica	12± 1.41	11±0.70	10±0.70	11±1.41
	Mixure	19± 0.00	35±0.00	17±2.82	21±0.70
Leave	*A. digitata*	11±2.12	11±1.41	10±0.70	11±2.21
	T. indica	12± 0.70	10±0.00	9±0.70	12±0.70
	Mixure	18± 0.00	23±0.00	15±0.00	20±0.70
Bark	*A. digitata*	11±0.00	9±0.70	10±0.70	10±0.00
	T. indica	12± 0.70	9±1.41	10±0.00	9±1.41
	Mixure	16±0.00	12±0.70	19±0.70	12±0.70

*M. D. I . Z ., Mean diameter of growth inhibition zone in mm.

higher activity (61.66%) than Baobab (15.69 %), whereas the bark showed 51.48 and 66.90% in Tamarind and Baobab respectively. Several reports indicated that the antioxidant potential of medicinal plants may be related to the concentration of their phenolic compounds which include phenolic acids, flavonoids, anthocyanins and tannins (Djeridane et al., 2006; Wong et al., 2006). The health benefit of fruits are mainly attributed to phenolic compounds and vitamins, which enhance their antioxidant, anticancer, anti-mutagenic, antimicrobial, anti-inflammatory and neuroprotective properties. Bio-guided fractionation of extracts might promote the development of alternative therapeutic compounds for the prevention and treatment of various diseases and disorders.

Anti-inflammatory activity

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. The results showed both ethanolic extracts leaves parts represent the highest anti-inflammatory activity even in mixture form, followed by fruits, then bark, while in individual manner bark extracts represent higher activity than fruits as shown in Table 3. The results showed a concentration-dependent inhibition of protein (albumin) denaturation by *A. digitata* and *T. indica* extracts. *T. indica* extracts expressed a higher activity compared to *A. digitata*. Previous study by Osman and Idrees (2017) reported that both *A. digitata* and *T. indica* extracts exerted marked anti-inflammatory properties.

Antimicrobial activity

Antibacterial activity

Diseases such as bacterial, fungal and infectious parasitic are mainly controlled by chemotherapeutics and antibiotics in aquaculture. Nevertheless, the uses of antibiotics and chemotherapy have been strongly criticized as they created problems with drug resistance bacteria (Harikrishnan et al., 2011). All tested extracts showed various degrees of biological activity on the tested pathogens. All plants parts combinations showed remarkable activity against tested bacteria and fruit mixtures showed the highest activity against *B. subtilis* (19 mm), *S. aureus* (35 mm) and *S. typhi* (21 mm), while the mixture of bark extracts showed high activity against *E. coli* (19 mm). The results are presented in Table 4. Plant extracts are an important part in agroecology, as they benefit the environment in combating pathogenic organisms, without resorting to synthetic chemicals. (Rivera et al., 2014). Previous study showed that the significant differences observed in antibacterial activities

Table 5. Antifungal activity of parts extracts against *C.albicans* and *A.niger.*

Part of plant	Sample	*Fungi* (M.D.I.Z)*	
		C. albicans	***A. niger***
Fruit	*A. digitata*	15±1.41	13±0.70
	T. indica	18±0.70	16±0.70
	Mixure (1:1)	32±0.70	28±0.70
Leaves	*A. digitata*	16±0.70	21±0.70
	T. indica	13±1.41	17±0.00
	Mixure (1:1)	28±1.41	36±0.00
Bark	*A. digitata*	20±0.00	14±0.70
	T. indica	15±1.41	16±1.41
	Mixure (1:1)	30±0.00	33±1.41

*M. D. I . Z ., Mean diameter of growth inhibition zone in mm.

suggest that extract mixtures affect in a different way each of the tested food-borne pathogen bacteria; while differences among extract mixtures suggest that at least one extract mixture affect in a different way the bacterial growth. *A. digitata* leaves contain active ingredients against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *S. typhi*, *C. albicans, A.niger*, and *P. rotatumat* (Abiona et al., 2015).

Antifungal activity

All extracts and blends of the two plants were tested for their antifungal activity and the results are shown in Table 5. The inhibitory percentage of extracts and their combinations against *C. albicans* ranged from 13-32 mm and 13-36 mm against *A. niger*. The blends of all plant extracts of the same part showed higher activity than original extracts of the same plant.

Remarkable synergistic effect of plant extracts was observed because the plants have the same pharmacological activity, and the active compounds present in each extract act together to targeted receptors, therefore producing higher effect than using any plant extract alone. From other point of view, some of the plants compounds may not have pharmacological activity but act as a potentiated agents to improve the activity of the compounds found in the other plant extract with pharmacological activity (Atanasov et al., 2015).

Conclusion

In this study different part of *A. digitata* and *T. indica* were used. The plants are rich sources of chemical and bioactive compounds including therapeutic and dietary constituents. The present study suggests that blending of the plants parts of *A. digitata* and *T. indica* could be a potential source of natural antioxidant and anti-inflammatory properties that could have great importance in the inhibition of inflammation as well as against bacterial and fungal infections. The findings of this study suggest that the tested plants and their combinations can be developed as effective herbal phyto-pharmaceutical drugs for treatment and nutrition. Based on the research outcomes we recommend conducting more experiments on the combination such as anticancer activity, bacterial and fungal infections and virus infections.

CONFLICT OF INTERESTS

The authors have not declared any conflict of Interests.

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