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Antioxidant and antimicrobial activity of leaves of *Terminalia catappa* and *Anacardium occidentale*: A comparative study

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Abstract
Medicinal plants serves as an important component of the healthcare system in India. A large number of medicinal plants and their purified constituents have shown beneficial pharmaceutical potentials. The quantity and quality of phytochemicals present in different plants differ from one another. In fact, the biological activity of different plants is related to the difference in distribution of bioactive compounds which are more frequent in some plants than in others. Therefore, the present study is to compare the antibacterial and antioxidant activities of methanolic leaf extract of *Terminalia catappa* and *Anacardium occidentale*. The antioxidant activity of methanolic extracts of *T. catappa* and *A. occidentale* were determined using DPPH reagent. The methanolic extract of *A. occidentale* leaf shows the most effective scavengers of DPPH radicals than *T. Catappa* leaf. The test extracts of *T. catappa and A. occidentale* were able to inhibit all the bacterial strains studied viz *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus cereus, Zymomonas mobilis, Serratia marcescens.*

Keywords: *Terminalia catappa*, *Anacardium occidentale,* Antioxidant property, Antibacterial activity, DPPH.

1. Introduction
Medicinal plants have the ability to synthesize a wide variety of chemical compounds that play a major role in primary health care as therapeutic remedies and also serve as an alternative source for western medicine. Chemical compounds in plants mediate their effects on the human body through processes identical to those in conventional drugs [1]. In traditional practices plant based preparations are the major medicine for the treatment of infectious diseases and they have very low level risk compared to presently available synthetic drugs [2]. Secondary metabolites present on the plant possess various interesting biological activities [3]. These compounds differ widely in terms of structure, biological properties and mechanisms of actions. These secondary metabolites include flavonoids, phenols, phenolic glycosides, saponins, and glycosides [4, 5].

Many research studies have demonstrated that medicinal plants contain various components with antioxidant and anti-microbial activity, which are responsible for their beneficial health effects. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [6]. Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular and neurodegenerative diseases [7, 8]. Antioxidant agents of natural origin have attracted special interest because they can protect the human body from free radicals [9, 10]. Phenolic compounds possess a wide spectrum of biological effects including antioxidant and free radical scavenging [11, 12].

Indian Almond (*Terminalia catappa)* family is a large tropical tree in the leadwood family, combretaceae that grows mainly in the tropical regions of Asia, Africa and Australia. The leaves and barks are mostly used due to their diaphoretic, anti-indigestion, hepatoprotective, antiperoxidation, antisickling and anti-dysentery effects [13, 14, 15, 16, 17]. The aqueous extract of the young leaves are used against headache and colic [18] while the bark is used as an astringent in dysentery and thrush [19]. Antibacterial, antifungal, analgesic, anticolic, antihyperalgesic and anti-inflammatory properties of seed oil have also been reported [20, 21].

Anacardium occidentale L. (cashew nut), belonging to Anacardiaceae family, and a native of Brazil, having great economic and medicinal value and which is composed of some 60 to 74 genera and 400 to 600 species. *Anacardium occidentale L.* leaves, stem and bark extracts are utilized widely for the treatment of diarrhea, dysentery and colonic pain [22]. It has also been reported to possess anti-diabetic, anti-bacterial, anti-inflammatory and anti-ulcerogenic [23]. The leaves are also used in Brazil for eczema, psoriasis, scrofula, dyspepsia, genital problems, and venereal diseases, as well as for impotence, bronchitis, cough, intestinal colic, leishmaniasis, and syphilis-related skin disorders.

2. Materials and Methods

2.1 Preparation of extracts

The mature leaves of *Terminalia catappa* and *Anacardium occidentale* collected locally were washed with water, shade dried and grounded into a fine powder. 60 g of the dried leaf powder was extracted with methanol using soxhlet apparatus for 8 hours. After extraction, it was filtered and evaporated by using a rotary evaporator. The extracts obtained were stored at 4 ºC.

2.2 Determination of Antibacterial activity

2.2.1 Test Microorganism

Stock cultures of *Staphylococcus aureus* (MTCC 96), *Bacillus cereus* (MTCC 430), *Escherichia coli* (MTCC 198), *Salmonella typhi* (MTCC 733), *Serratia marcescens* (MTCC 2708) and *Zymomonas mobilis* (MTCC 93) were obtained from the Microbial Type Culture Collection and gene bank (MTCC), Institute of Microbial Technology, Chandigarh, India. The cultures were stored on nutrient agar slants at 4°C temperature. Overnight broth cultures were used for testing the antibacterial activity.

2.2.2 Agar disc diffusion method

The antibacterial sensitivity testing of the extract was determined using disc diffusion method. A single concentration of 500 mg of two plant extracts were dissolved in 1 ml methanol were used for the antimicrobial activity. 100 µl of the inoculated microorganisms from nutrient broth were spread over the plates containing Mueller-Hinton agar using sterile cotton swabs. The sterile discs having a diameter of 6 mm were impregnated with 30 µl of a leaf extract (*T. catappa, A. occidentale*) and were placed on inoculated surface of agar plate with the help of sterile forceps. Streptomycin antibiotic disc were placed in the middle of the plate as a positive control. The plates were then incubated according to the incubation period of each microorganism for 24hr to observe the zone of inhibition produced by the extracts.

2.3 Determination of Antioxidant activity

2.3.1 DPPH radical scavenging assay

The ability of the plant extract to scavenge 1,1-dyphenyl-2-picrylhydrazyl (DPPH) free radicals was assessed by the method described by Tekao *et al.* [24]. DPPH (20 mg) was dissolved in methanol (250 mL) to obtain the concentration of 80 µg/mL. The stock solution of the plant extract was prepared in methanol to achieve the concentration of 1 mg/mL. Dilutions were made to obtain concentrations of 1000, 750, 500, 250 µg/mL. Ascorbic acid was used as standard in 1-100 µg/ml concentration. 1 ml of the diluted plant extract was mixed with 1 ml of DPPH. After 30 min in darkness at room temperature (25 ºC), the absorbance was recorded at 517 nm. The control samples contained all the reagents except the extract. The percentage inhibition was calculated using the following equation,

$$\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

3. Results and Discussion

3.1 Antibacterial activity

The methanol extract of *T. catappa* showed maximum antimicrobial activity against *Staphylococcus aureus* with a zone of inhibition 20 mm and minimum antimicrobial activity against *Escherichia coli* with a zone of inhibition 11mm. The extract showed comparatively high antimicrobial activity against *Zymomonas mobilis* with a zone of inhibition of 18mm. *Bacillus cereus* and *Salmonella typhi* showed a similar range of antimicrobial activity with a zone of inhibition 15mm. *Serrratia macescens* was found its antimicrobial activity with a zone of inhibition 12mm (**Table 1).**

Table 1: Antimicrobial activity of *T. catappa* and *A. occidentale* against different bacteria.

SL. NO	BACTERIA	*T. catappa* Zone of inhibition (mm)	*A. occidentale* Zone of inhibition (mm)	ANTIBIOTIC DISC (standard-streptomycin) Zone of inhibition (mm)
1	*Staphylococcus aureus*	20	12	20
2	*Bacillus cereus*	15	13	30
3	*Escherichia coli*	11	18	25
4	*Salmonella typhi*	15	14	25
5	*Serratia marcescens*	12	15	20
6	*Zymomonas mobilis*	18	16	27

A. occidentale showed antimicrobial activity against six human pathogens. It showed maximum antimicrobial activity against *Escherichia coli* with a zone of inhibition of 18mm. *Zymomonas mobilis* also showed a high range of antimicrobial activity with a zone of inhibition 16mm. The methanol extract showed a zone of inhibition of 15mm against *Serratia marcescens. Salmonella typhi* showed antimicrobial activity with a zone of inhibition 14mm.The least activity was shown against *Staphylococcus aureus* with a zone of inhibition of 12mm for its antimicrobial activity.

The test extracts of *T. catappa and A. occidentale* showed interesting results, both extracts were able to inhibit all the bacterial strains studied viz *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus cereus, Zymomonas mobilis, Serratia marcescens.* The antibacterial activity of a plant extracts depends on the available bioactive compounds, secondary metabolites like Tannins, Saponins, Terpenoids, Alkaloids and Flavonoids [25]. Therefore, further isolation and identification of specific bioactive molecules from tested plant extracts could be a valuable alternative for the synthetic antimicrobial drugs. The appreciable quantities of phytochemicals present in the leaf extracts were responsible for the high antibacterial effect observed.

3.2 Antioxidant activity

The antioxidant activity of methanolic extracts of *T. catappa* and *A. occidentale* were determined using methanol solution of DPPH reagent. DPPH is a very stable free radical. The effect of an antioxidant on DPPH radical scavenging is due to their hydrogen donating ability or radical scavenging activity. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form diphenylpicrylhydrazine with the loss of its violet color.

The antioxidant activity of the plants *T. catappa* and *A. occidentale* were represented in **figure 1**. The results of antioxidant activity revealed that both the methanolic plant extracts showed antioxidant activity. Among the two extracts tested for the *in vitro* antioxidant activity using the DPPH method, the crude methanolic extract of *A. occidentale* showed more antioxidant activity. *A. occidentale* showed a percentage inhibition of 94.43µg/ml and *T. catappa* showed a percentage inhibition of 91.98µg/ml for 1000µg/ml concentration. The results indicate that the antioxidant activity of the crude extract of both the plants is lower than that of the standard.

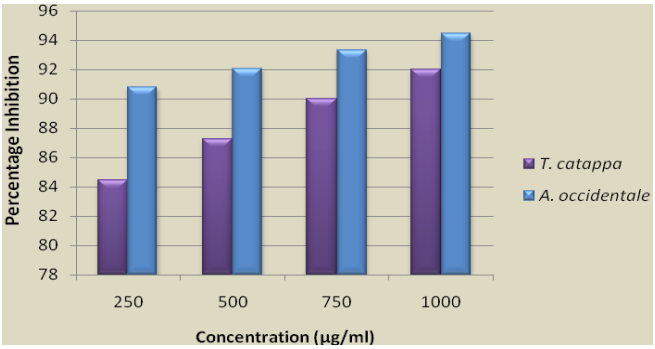

Fig 1: Graph showing the antioxidant property of the methanolic extract of *T. catappa* and *A. occidentale*

Antioxidants are chemical molecules capable of scavenging free reactive species of oxygen and nitrogen, which are continually produced in the metabolic cells in response to stress caused by atrophy, necrosis, anorexia and infections. Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. Young *et al.* [15] stated that the antioxidant and hepatoprotective properties of the extract of *Terminalia catappa* have been use *in vivo* to resolve oxidants generated by PMN-stimulated leukocytes.

4. Conclusion

Based on the results it have been validated that the methanolic extracts of *T. catappa* and *A. occidentale* have potent antimicrobial and antioxidant properties along with therapeutic potential and may play an important role in drug development and also in the development of health supplements. The present study thus scientifically strengthens the candidature of *T. catappa* and *A. occidentale* leaves in the preparation of medicinal aids against diseases arising due to oxidative stress and against various pathogens.

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