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Phytochemical, Antioxidant and Antibacterial Studies on *Bambusa arundinacea* and *Mangifera indica*

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ABSTRACT

This study was formulated to check the phytochemical, antioxidant, antibacterial potential of Bambusa arundinacea (Bamboo) and Mangifera indica (Mango) trees. Aqueous, ethanolic and methanolic extracts were prepared from leaves of former and stem bark of later. The phytochemical screening of the extracts showed the presence of various bioactive compounds such as carbohydrates, flavonoids, saponins and proteins in B. arundinacea, alkaloids, flavonoids, tannins, saponins, steroids and cardiac glycosides in M. indica. Total phenolic concentration and percentage of free radical scavenging activity was more in ethanolic extracts of B. arundinacea and M. indica followed by methanolic extracts and aqueous extracts. Highest percentage of ferric reducing antioxidant power was found in ethanolic extracts and lowest in aqueous extracts indicates that ethanolic extracts has more antioxidant potential than the other two extracts. Ethanolic extracts of both plants had higher inhibition on the tested Gram positive (B. subtilis & S. aureus) as well as Gram negative (E.coli & P. aeruginosa) bacteria evidenced from the zones of inhibition. M. indica showed more therapeutic potential as compared to B. arundinacea and ethanolic as well as methanolic extracts of both the tested plants were more effective than aqueous extracts due to better extraction power of organic solvents. Overall study indicates that B. arundinacea and M. indica are potential source of natural antioxidants, phytochemicals and antibacterials that can be used for the development of novel drugs and may represent new source of antimicrobials with stable, biologically active components that can establish a scientific base for further use in modern medicines.

Keywords: *Antioxidant, Flavonoids, Methanolic, Therapeutic, Phytochemical*

INTRODUCTION

In Indian scenario, World Health Organization (WHO) estimates about 70-80% of Indians depend on Indian system of medicine like Unani, Siddha, and Ayurvedha[1]. Traditional use of herbal medicine is usually an integral part of culture around the world, which has been used in medical practice for thousands of years and has made a great contribution for maintaining human health before spread of modern science[2]. Although modern medicine may be available in developed and developing countries, herbal medicine has often maintained popularity for historical and cultural reasons. Concurrently many people in developed countries have begun to turn to alternative or complementary therapies including medicinal herbs. Detailed knowledge of both of these factors has become available only fairly recently, but clues to plants worthy of investigation are provided very often by the study of plants used traditionally in traditional medicine[3].The emerging importance of biologically active medicinal plants and their constituents as possible therapeutic measures has become a subject of active scientific investigation. It is likely that in future safe and effective medicines will be developed from medicinal plants to treat various degenerative diseases[4].

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Many pharmaceutical companies show interest in plant derived drugs mainly due to the current widespread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs, which have adverse side effects. Free radicals are generated in our body during the normal metabolic processes and during exposure to adverse patho-physiological conditions and cause damage to DNA[5], which is associated with the process of carcinogenesis. Phytochemicals such as phenolics, carotenoids and dietary fibers are gaining increased attention because of their antioxidant, anticarcinogenic, antimutagenic, and other health promoting properties[6].

Studies have been carried out to determine antioxidant properties of various plants for the development of herbal medicine and nutritional supplementation in neutraceuticals. Much attention has been focused in finding naturally occurring antioxidants because they are biodegradable non-toxic products that replace synthetic antioxidants because of their harmful/ adverse side effects. *Bambusa arundinacea* locally known as bamboo or bans are popularly known for their industrial uses. Being low in fat content and high in potassium, carbohydrate, dietary fibres, vitamins and active materials, bamboo shoots are consumed in raw, canned, boiled, marinated, fermented, frozen, liquid and medicinal forms[7]. A number of studies of bamboo have yielded information about the chemical constituents, but no systematic evaluation has been carried out, so it is difficult to determine which of the identified compounds might be among the primary active constituents[8]. The shoots are reported to have anticancer, antibacterial, and antiviral activity. Shoots have antioxidant capacity due to the presence of phenolic compounds[9]. *Mangifera indica*, also known as mango (aam), important herb in the Ayurvedic is used to cure monorrhagia, leucorrhoea, bleeding piles and in case of haemorrhage from nose[10]. Several studies have reported polyphenolic compounds in mango flesh and peel, including various ascorbic acids, dehydroascorbic acids, flavonoids, xanthones, phenolic acids, and gallotannins[11,12]. Keeping in mind the various applications and uses of *B. arundinaceae and M. indica* the present study was designed to access the antioxidant and antibacterial activities along with phytochemical analysis of extracts of *B. arundinaceae and M. indica* in order to gain a new source of bioactive compounds for the development of novel therapeutic agents.

MATERIALS AND METHODS

Collection of Plant Material

The mature leaves of *B. arundinacea* were collected from a nursery at Fazilka, Punjab and bark of *M. indica* was obtained from Kotla in Kangra (Himachal Pradesh), then cleaned properly with normal water and then 4-5 times with distilled water, dried under shade for 7-8 days at normal room temperature. Then samples were pounded with mortar and pestle into small particles and blended to powder using an electric blender.

Test Organisms

Two Gram negative bacteria *Escherichia coli* (MTCC-4438), *Pseudomonas aeruginosa* (MTCC-424) and two Gram positive bacteria *Staphylococcus aureus* (MTCC-497) and *Bacillus subtilis* (MTCC-1427) used to check the antibacterial activity of *B. arundinacea* and *M. indica* were procured from Institute of Microbial Technology (IMTECH) Chandigarh.

Preparation of Extracts

Aqueous, ethanolic and methanolic extracts of *B. arundinacea* leaves and *M. indica* bark powder were prepared by dissolving 10gm powdered sample in 100 ml distilled water, ethanol and methanol in three different flasks and then mixed thoroughly by shaking. After 24 hours, suspensions of all the three flasks were filtered using a Soxhlet apparatus, filtrates were concentrated at 50˚C for 30 minutes and stored in air tight bottles for further use.

Phytochemical Analysis of Extracts

Biologically active plant chemicals other than nutrient components that have a beneficial effect on health of human are termed as phytochemicals[13]. Phytochemicals provide plants with color, flavor and natural protection against pests. The extracts were subjected to chemical tests to identify the phytochemical constituents such as carbohydrates, reducing sugars, proteins & amino acids, flavonoids, saponins, glycosides, tannins, alkaloids and steroids[14].

Antioxidant Activity

Antioxidants can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. The antioxidant activity of each extract was tested using the following methods:

Total Phenolic Content

The amount of total phenol in extracts was determined with Folin Ciocalteau reagent with slight modification[15]. Gallic acid was used as the reference standard, and the results were expressed as mg of gallic acid equivalent (GAE)/100gm sample in fresh weight.

DPPH Radical Scavenging Activity

This is the simplest method, wherein the prospective compound or extract is mixed with DPPH solution and absorbance is recorded after a defined period. Preparation of DPPH solution was adopted from Blois[16] with minor modification. Each extract 50 µg/ml was pipetted into DPPH solution concentration 50µg/ml (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was read at wavelength 517 nm by using UV-Vis spectrophotometer. Methanol was used as a blank and DPPH solution 50 µg/ml as standard. Analysis was done in triplicate for standard and each extract. Antioxidant activities of each extract were determined based on the reduction of DPPH absorbance by calculating percentage of antioxidant activity[17].

FRAP Method (Ferric Reducing Antioxidant Power)

FRAP is used to measure the total antioxidant power of extracts. FRAP solution was prepared in acetate buffer pH 3.6. Each extract (50µg/ml) was pipetted into FRAP solution 50 µg/ml (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was read at wavelength 593 nm by using UV-Vis spectrophotometer. Acetate buffer was used as a blank and FRAP solution 50µg/ml was used as standard. Analysis was done in triplicate for standard and each extract. Antioxidant capacity of each extract was determined based on increase in Fe (II) - TPTZ absorbance by calculating percentage of antioxidant capacity[18].

Antibacterial Activity

Antibacterial activity was determined by Disc Diffusion Method[19]. When a filter paper disc impregnated with extract placed on agar, the extract will diffuse from the disc into the agar. If an organism is spread on the agar plate then it will not grow in the area around the disc if it is susceptible to the extract. This area of no growth around the disc is known as zone of inhibition.

RESULTS AND DISCUSSION

To access the antioxidant and antibacterial activities along with phytochemical analysis of *Bambusa arundinacea* and *Mangifera indica* extracts were prepared from leaves of *B. arundinacea* and bark of *M. indica* using water, ethanol and methanol as solvents following standard procedures.

Phytochemical Analysis of Extracts

Phytochemicals exhibit a wide range of beneficial biological effect and could neutralize oxidation of biological molecules by scavenging free radicals and chelating free catalytic metals. These phytochemicals possess antioxidant activity. Natural products belonging to saponins, flavonoids, proteins and amino acids were shown to be present in all the extracts of both the plants. However, reducing sugars and cardiac glycosides were absent in extracts of *B. arundinacea* and carbohydrates were absent in extracts of *M. indica.* Cardiac glycosides are known to possess serious toxicity because they could affect the heart and atrial fibrillation[20]. Absence of cardiac glycosides in *B. arundinacea* makes it potential and safe herbal supplement.

Saponins are mild detergent, used in hyperchloles-trolaemia, hyperglycaemia, weight loss etc. and also used as antioxidant, anti-cancer & anti-inflammatory agent. Tannins are reported to exhibit antiviral, antifugal, antibacterial, anti-tumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectivity and also used as diuretic. Plant tannins have been recognized for their pharmacological properties and are known to make trees and shrubs a difficult meal for many caterpillars[21].

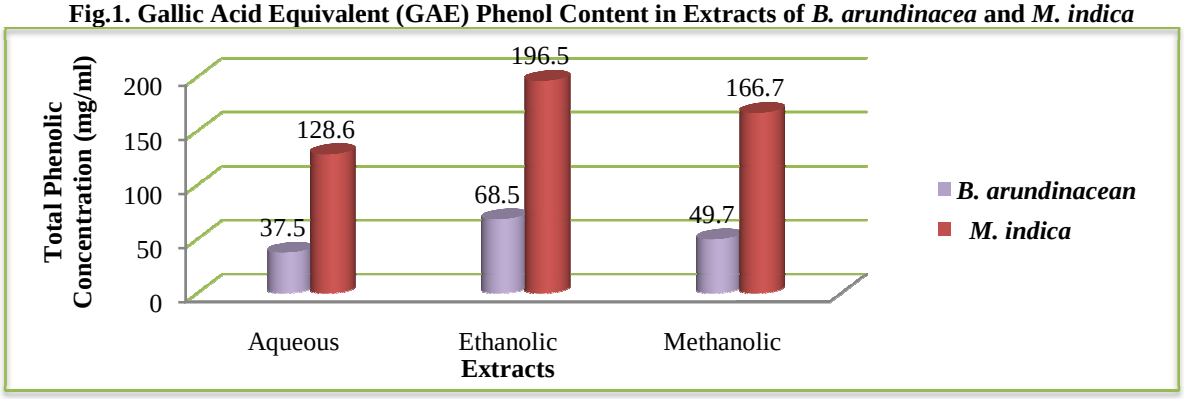
Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergies, virus and carcinogens. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity[22].

Antioxidant Activity

Total Phenolic Content

Ethanolic extracts of *B. arundinacea* and *M. indica* were rich in the phenolic content (68.5mg/ml and 196.5mg/ml respectively) followed by methanolic extracts (49.7mg/ml and 166.7mg/ml respectively) and aqueous extract (37.5mg/ml and 128.6mg/ml respectively) indicated that ethanolic extract has more antioxidant capacity than methanolic and aqueous extracts (Fig. 1).

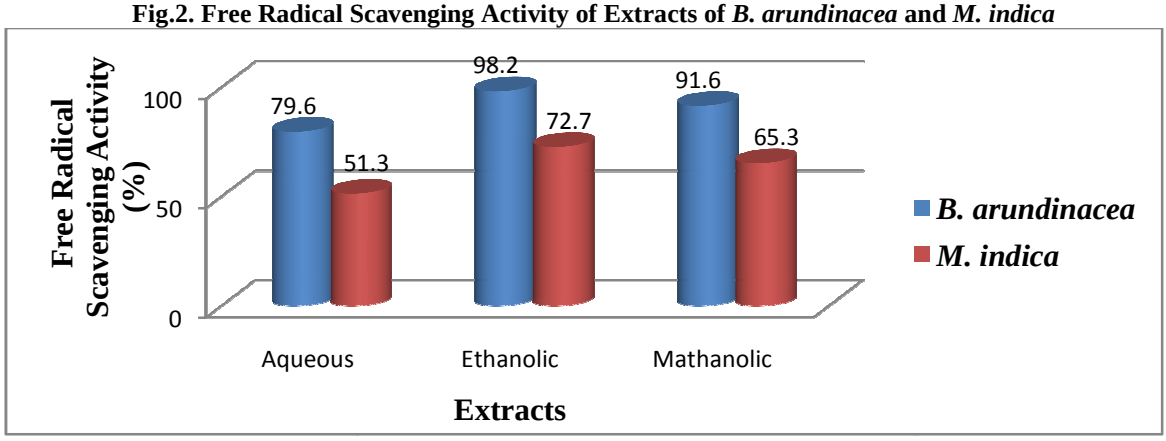
Fig.1. Gallic Acid Equivalent (GAE) Phenol Content in Extracts of *B. arundinacea* and *M. indica*



DPPH Radical Scavenging Activity

Ethanolic extracts of *B. arundinacea* and *M. indica* showed highest DPPH free radical scavenging activity (98.2% and 72.7% respectively) and aqueous extracts showed lowest (79.6% and 51.3% respectively) i.e. ethanolic extracts were found to have higher antioxidant activity than methanolic extracts and aqueous extracts thus prevent the formation of free radicals more efficiently which causes various diseases (Fig.2). The results of the present study were found to be very close to earlier studies where DPPH radical scavenging activity was found to be around 95% in *Bambusa vulgaris* 'Vittata' methanolic leaf extract and the methanolic extract showed highest free antiradical scavenging activity as compared to aqueous and butanolic extract of bamboo leaves[23,24].

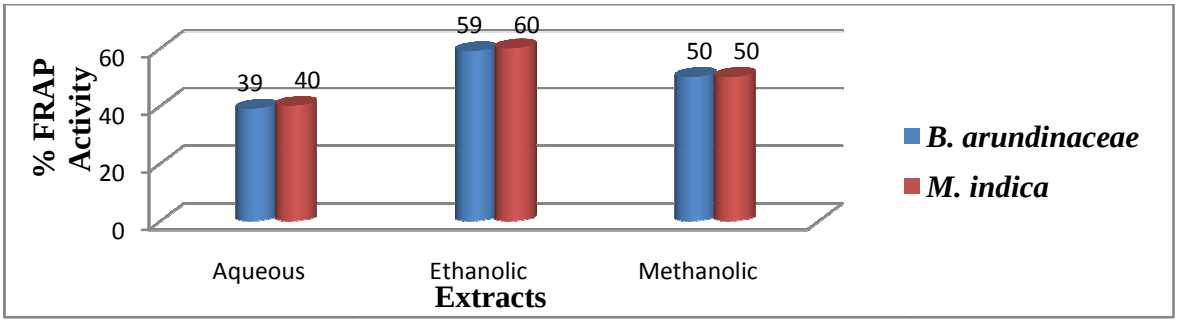
Fig.2. Free Radical Scavenging Activity of Extracts of *B. arundinacea* and *M. indica*



FRAP Method (Ferric Reducing Antioxidant Power)

Ethanolic extracts of *B. arundinaceae* and *M. indica* showed highest (59% and 60% respectively) ferric reducing antioxidant power and aqueous extracts lowest (39% and 40% respectively) (Fig. 3). Results revealed that ethanolic extracts has more antioxidant power as compared to methanolic and aqueous extracts of bamboo leaves and mango stem bark. It indicates that ethanolic extracts has more ability to reduce the TPTA Fe (III) complex to TPTZ Fe (II) complex than the other extracts.

Fig.3. Ferric Reducing Antioxidant Power of *B. arundinaceae* and *M. indica* Extracts



It was earlier noticed that ferric reducing antioxidant power was comparatively higher for methanolic extract than aqueous and butanolic extract in *B. arundinaceae* leaves extract[24]. Results of phytochemical analysis of extracts of *Mangifera indica* were similar with results of earlier studies[25,26]. Total phenolic concentration of aqueous extract was 128.6mg/ml that resembles to the results of previous study where total phenolic concentration of *M. indica* was 128.20±22.00mg/ml[27].

Antibacterial Activity

The antibacterial activity was determined by measuring zone of inhibition (ZOI). The zone of inhibitions for tested bacteria showed that bacteria were more susceptible to ethanolic and methanolic extracts but found to be resistant to aqueous extract to some extent. Infact, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed maximum inhibition zones with ethanolic extracts (Fig. 4 & 5). Abubakar also showed that ethanolic extract was more effective for all organisms as compared to methanolic and aqueous extract of mango bark[28].

The results revealed that all extracts of *B. arundinacea* showed effective inhibitory action against *S. aureas* and these results were in concordance with earlier study by Singh *et al* [29]. Results of antibacterial activity of extracts of *Mangifera indica* stem bark were found to be similar with results of previous studies[30,31].

Fig.4. Antibacterial Activity of Extracts of *B. arundinacea*

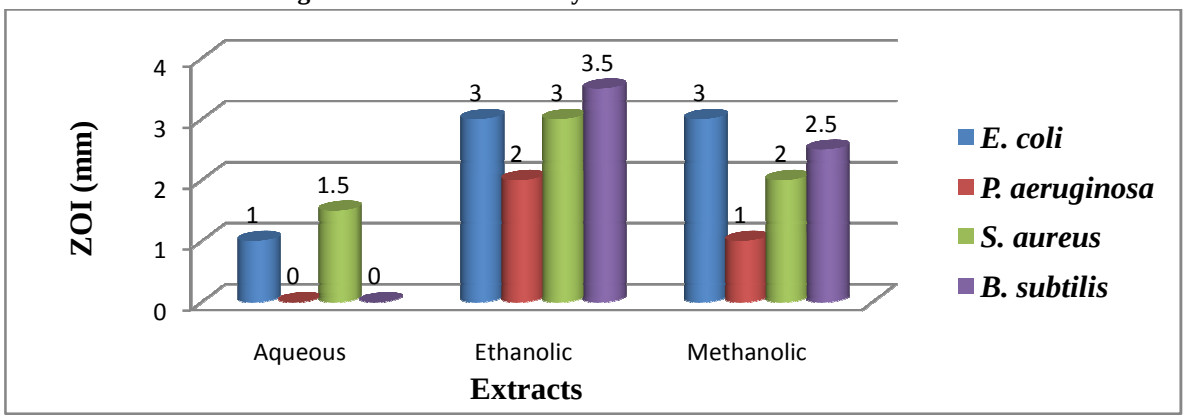
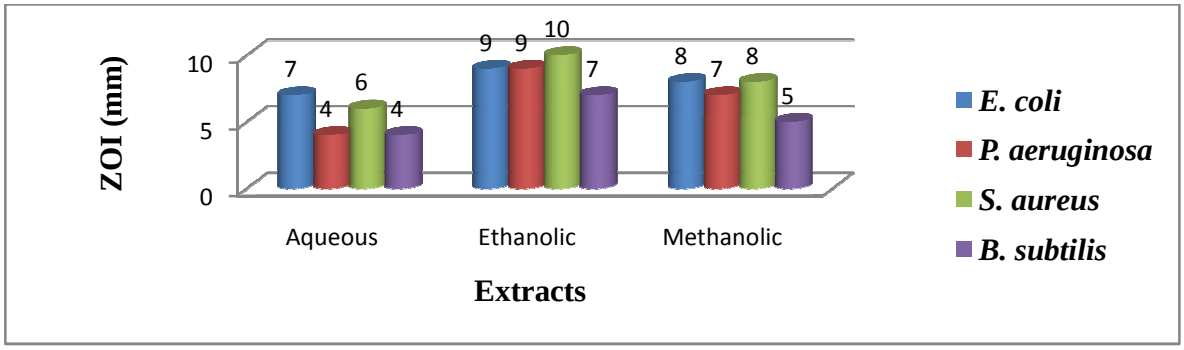


Fig.5. Antibacterial Activity of Extracts of *M. indica*



CONCLUSIONS

In conclusion it can be said that *Bambusa arundinacea* and *Magnifera indica* were found to contain a noticeable amount of total phenols and flavonoids, which play a major role in controlling oxidation. The presence of most general phytochemicals might be responsible for their therapeutic effects that further reflects a hope for the development of many more novel therapeutic agents in future for the production of synthetically improved therapeutic agents. These plants can be used as antibacterial agents against multiple drug resistant bacteria and to prevent the formation of free radicals, that further causes very dangerous diseases which otherwise are unable to be treated. However further investigations are necessary to identify the responsible components needed for their effective role as therapeutic plant and additional work should be done to fractionate the extracts to elicit a better understanding of the unique mixture of plant antioxidants and to determine the mechanism behind the antioxidant activity of these plants.

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