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PHYTOCONSTITUENTS AND ANTIBACTERIAL ACTIVITY OF *MORINDA TINCTORIA*

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ABSTRACT

The genus Morinda tinctoria plants have different uses; some are used as foods, and some species as remedy in traditional medicine. The powdered plant material was extracted by using four different solvents namely water, ethyl acetate, chloroform and methanol. The result indicates the presence of alkaloids, glycosides, saponins, tannins, flavanoids, cardiac glycosides, steroidal terpenes, anthraquinones and carbohydrates, in all four extracts of Morinda tinctoria. The chloroform extract showed highest antibacterial activity followed by ethyl acetate, Methanol and water and all the extracts showed maximum inhibition at the concentration of 200 µg/ml. Among the tested bacterial strains, Staphylococcus aureus showed high susceptibility to all the tested leaf extracts of Morinda tinctoria.

Keywords: Morinda tinctoria, Rubiaceae, phytoconstituents, antibacterial activity.

INTRODUCTION

Plants are the reservoirs of a large number of imperative organic compounds and they have long been used as the sources of medicines. Dependence on plants is prevalent in developing countries where the traditional herbal medicine plays a major role in health care and in the treatment of many infectious diseases. The rural population of a country is more disposed to traditional ways of treatment because of its easy availability and cheaper cost.

Antibiotic resistance has become a global concern (Westh, *et al*., 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow, *et al*., 2003). There is a continuous and urgent need to discover

new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas, *et al*., 1992). The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs (Latha and Kannabiran, 2006). It is therefore very necessary that the search for newer antibiotic sources be a continued process. Plants are the cheapest and safer alternative sources of antimicrobials (Pretorious and Watt, 2001; Sharif and Banik, 2006; Doughari *et al*., 2007). It has been suggested that the aqueous and ethanolic extracts from plants used in allopathic medicine are potential source of antiviral, anti-tumoral and antimicrobial agents (Chung, *et al*., 1995). Interest in large number of traditional natural products has increased (Taylor *et al*., 1996). The use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community (Sharif and Banik, 2006, Kubmarawa *et al*., 2007). The advent of science into the search for antibiotics largely depends on some of these medicinal plants as raw materials. Numerous studies have identified compounds within herbal plants that are effective antibiotics. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics; some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes. Hence, in the present study the phytoconstituents and antimicrobial activity of *M.tinctoria* is explored.

Morinda tinctoria, commonly known as Aal or Indian Mulberry is a species of flowering plant in the family Rubiaceae, native to southern Asia. It is an evergreen shrub or small tree growing to 5-10 m tall. The leaves are 15-25 cm long, oblong to lanceolate. The flowers are tubular, white, scented, about 2 cm long. The fruit is a green syncarp, 2-2.5 cm diameter. This paper explores the antimicrobial activity of *Morinda tinctoria*

MATERIALS AND METHODS

Collection of the plant material

Fresh leaves of *Morinda tinctoria* were collected and washed under running tap water. Then it was air

dried for more than a month. The dried leaves were then grind into coarse powder using the grinding machine. Then, the powder was filtered through the muslin cloth. The obtained fine powder was stored in the air-tight container till use.

Extraction of the plant material

About 10 gram of the powdered plant material was weighed and mixed with 25 ml of four different solvents namely water, ethyl acetate, chloroform and methanol separately in four different conical flasks. The contents were mixed well by occasional shaking as it had been kept on a rotary shaker for 30 minutes and left undisturbed for 24 hours. After 24 hours, the extracts were filtered through the cotton plug and then with the Whatmann No.1 filter paper and then solvent were removed with a rotary evaporator at low temperature and reduced pressure. The volume of the supernatant obtained were made upto 10 ml with the respective solvents.

PHYTOCHEMICAL SCREENING

The freshly prepared extracts were subjected to standard phytochemical analyses for different constituents such as tannins, alkaloids, flavonoids, anthraquinones, glycosides, saponins, terpenoids and reducing sugars as described by Jigna et al. (2006) and Harbourne (1998). This was done on the different extracts to ascertain the presence of bioactive components present in plant extracts.

Test for amino acids: One ml of the extract was treated with few drops of Ninhydrin reagent. Appearance of purple colour shows the presence of amino acids.

Test for anthraquinones: Five ml of the extract solution was hydrolysed with diluted Concentrated sulphuric acid extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink colouration suggested the positive response for anthroquinones.

Test for flavanoids : To one ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow colour was produced in the plant extract, which become colourless on addition of a few drops of dilute acid. This indicates the presence of flavonoids.

Test for glycosides: The extract was hydrolysed with HCL for few hours on a water bath. To the hydrolysate, 1 ml

of pyridine was added and a few drops of sodium nitroprusside was mixed and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Test for phytosterol: The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol. The residue was dissolved in few drops of diluted acetic acid ; 3 ml of acetic anhydride was added followed by few drops of concentrated sulphuric acid. Appearance of bluish green colour showed the presence of phytosterol.

Test for saponins: The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of saponins.

Test for steriods: One ml of the extracts was dissolved in 10 ml of chloroform and equal volumes of concentrated sulphuric acid was added by sides of the test tubes. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for tannins: 5 ml of the extract and a few drops of 1% lead acetate were added. A yellow precipitate was formed, indicates the presence of tannins.

Test for triterpenoids: Ten mg of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid. Formation of reddish violet colour indicates the presence of triterpenoids.

Test for alkaloids: To 5ml of the extract, 2ml of the hydrochloric acid was added followed by addition of either Wagner's reagent or Mayer's reagent. Presence of the alkaloids was indicated by the formation of the reddish brown precipitate or white creamy precipitate.

Test for phenolic compounds: The extract was mixed well with the 5ml of the distilled water then the solution was neutralized by adding 5% ferric chloride the dark green colour formed shows the presence of phenolic compounds.

Test for fixed oils and fats: To identify the presence of fixed oils and fats the precipitate obtained was pressed in between the two filter papers, appearance

of the oil stain on the paper shows the presence of fats.

Test for aromatic acids: Few drops of the extract, sodium bicarbonate solution was added and mixed well. The brisk effervescence was produced which shows the presence of the aromatic acids.

Test for coumarin: To 1ml of the extract, a few drops of dilute sodium hydroxide was added. A yellow colour was produced in the plant extract which indicates the presence of the coumarin.

Test for quinones: Appearance of the red colour on reaction of the extract with the few drops of the concentrated sulphuric acid confirms the presence of the quinones.

Test for gum and mucilages: The extract was diluted with the 10ml of the distilled water and 25ml of the alcohol which gives the white precipitate thus shows the presence of the gum and mucilages.

Test for cardiac glycosides: The test was carried out by treating 2ml of the filterate with 1ml of glacial acetic acid and few drops of the ferric chloride and concentrated sulphuric acid. Appearance of the greenish blue colour indicates the presence of the cardiac glycosides. This test was referred as the Keller Kilani test.

Test for volatile oils: 0.1ml of the dilute sodium hydroxide solution was mixed with the few drops of the extract then the solution was hydrolysed with the hydrochloric acid to produce the white precipitate which indicates the presence of the volatile oils.

Test for carbohydrates: The filterate was prepared by diluting 100mg of the extract with the 5ml of the distilled water, thus filtrate has undergone two reactions. When the filtrate was mixed well with the 2 drops of napthol and 1ml of concentrated sulphuric acid, it will lead to the formation of the violet colour ring. When the filterate was treated with the 1ml of the Barfoed's reagent and kept in a boiling water bath for 2minutes, the appearance of red precipitate confirms the presence of the carbohydrates.

PREPARATION OF THE TESTED ORGANISMS

Preparation of standard bacterial suspensions

In vitro anti-microbial activity was examined for four different extracts (water, ethyl acetate, chloroform and methanol). The microorganisms investigated were Streptococcus, Staphylococcus aureus and Pseudomonas aureginosa. All the bacterial strains were

maintained at 4°C on nutrient agar slants. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

ANTIMICROBIAL ASSAY

The bioassay used was the standard disk diffusion assay adapted from Taylor et al. (1995). Nutrient agar plates were prepared for doing bioassays against bacteria. The bacterial were inoculated from the respective 24 hrs broth by spread plate method. Under aseptic conditions empty sterilized discs (Whatmann No.1 filter paper 5-6 mm diameter) were impregnated with 10µl of different concentrations (50, 100, 150 and 200 µg/ml) of water, ethyl acetate, chloroform and methanol respectively using micropipettes and the residual solvents were completely evaporated. A standard disc containing streptomycin (10 microlitre) for bacteria and blank discs impregnated with respective solvents followed by evaporation were used as positive and negative control respectively.

All petridishes were sealed with the sterile laboratory parafilm to avoid eventual evaporation of the test samples. The plates were left for 30 minutes at room temperature to allow the diffusion of the extracts and then they were incubated at 37°c for 24 hours to allow the maximum growth of the microorganisms.

RESULTS

Table 1 Shows the presence of the various phytochemical constituents like alkaloids, glycosides, saponins, tannins, flavanoids, cardiac glycosides, steroidal terpenes, anthraquinones and carbohydrates.

The antibacterial activity of the four different extracts namely water, chloroform, methanol and ethyl acetate of the Morinda tinctoria leaves in the increasing concentration against Staphylococcus aureus, Streptococcus mutans and Pseudomonas aeruginosa were shown in Figure 1, Figure 2 and Figure 3 respectively

Of these extracts, chloroform showed highest antibacterial activity followed by ethyl acetate, chloroform and water, and all the extract showed maximum inhibition in the concentration of 200. The graph clearly shows the gradual increase in the inhibitory effect of the leaf extracts.

Depending upon the solvents used, the antibacterial activity of the leaf extract differs. Thus, the chloroform extract showed the maximum inhibition in all the concentrations followed by all other extracts.

Table 1: Phytochemical Analysis of *M.Tinctoria* Leaf extract with Different solvents.

S. No	***Name Of The Test***	***Water***	***Chloroform***	***Methanol***	***Ethyl Acetate***
1	Amino acids	-	-	-	-
2	Anthraquinones	-	+	+	+
3	Flavanoids	+	+	+	+
4	Glycosides	+	+	+	+
5	Phytosterols	-	+	-	+
6	Saponins	+	+	+	+
7	Steroids	+	+	+	+
8	Tannins	-	+	+	+
9	Triterpenoids	-	+	+	-
10	Alkaloids	-	+	+	+
11	Phenolic compounds	-	-	+	+
12	Fixed oils and Fats	-	+	+	-
13	Aromatic acids	-	-	+	-
14	Coumarin	-	+	-	-
15	Quinones	-	-	+	-

16	Gums and Mucilages	-	-	-	+
17	Cardiac glycosides	+	+	+	-
18	Volatile Oils	-	-	-	-
19	Carbohydrates	+	+	+	+

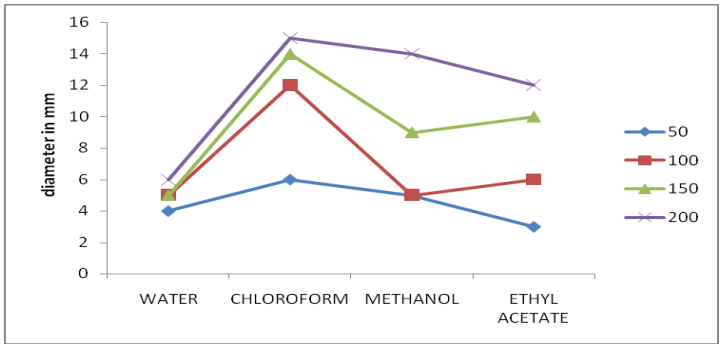

Figure 1: Antibacterial activity of Different Leaf Extract Against *Staphylococcus aureus*

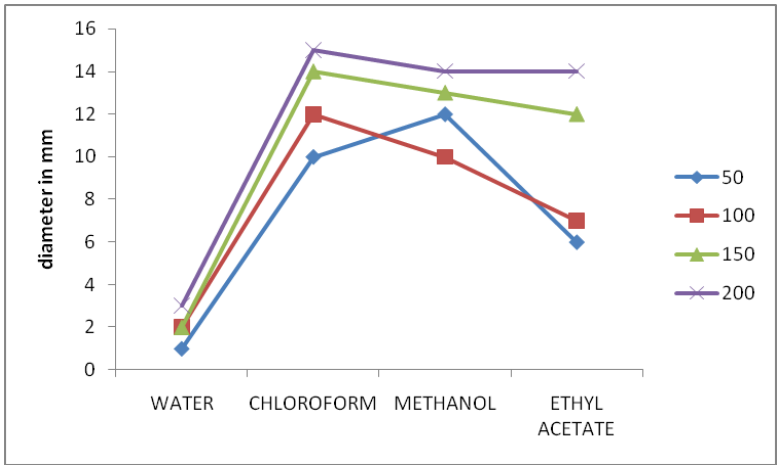

Figure 2: Antibacterial activity of Different Leaf Extract Against *Streptococcus mutans*

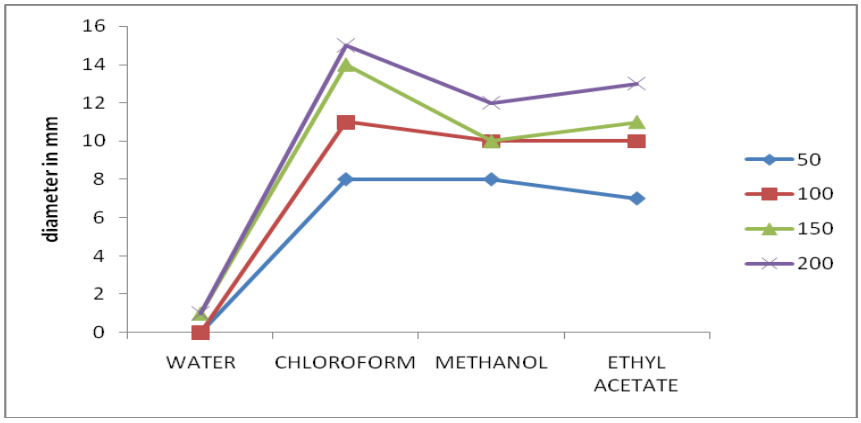

Figure 3: Antibacterial activity of Different Leaf Extract against *Pseudomonas aeruginosa*

Aqueous extract showed highest inhibition against the Staphylococcus compared to the other two bacteria. Chloroform also showed highest mean zone of inhibition against Staphylococcus whereas, in Streptococcus and Pseudomonas the mean zones are same. Against both the Staphylococcus and Streptococcus methanol extract showed maximum mean zone inhibition compared to that of the Pseudomonas. The ethyl acetate extract showed maximum activity against streptococcus followed by other two bacterial species.

DISCUSSION

Phytoconstituents have been found to inhibit bacteria, fungi, viuses and pests (Marjorie, 1999). In the present study, the leaves of M.tinctoria have been determined to possess antibacterial activity against staphylococcus, streptococcus and pseudomonas. Thus the presence of the phytoconstituents in the leaf extracts may be responsible for the antibacterial activity of the plant. Among the extracts tested, the chloroform and methanol extracts exerted highest activity on bacterial agents tested compared to the ethyl acetate and water extracts. The differences in the observed activities of the various extracts may be due to the varying degrees of solubility of active constituents in the four solvents used (De Boer et al., 2005). Activities of the various extracts were comparable to those of standard antibacterial agents streptomycin and ampicillin. Demonstration of the antibacterial activity against the test bacteria is an indication that there is possibility of sourcing alternative antibiotic substances in these plants for the development of newer

antibacterial agents. Successive isolation of botanical compounds from plant material is largely dependent on the type of the solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but this study shows the plant extract by chloroform and methanol provided more consistent antimicrobial activity compared to those extracted by water.

These observations can be rationalized in terms of polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. The growth media also seem to play an important role in the determination of the antibacterial activity (Lin et al., 1999). The results of the present study supports the traditional usage of the studied plants and suggests that some of the plant extracts possess compounds with antimicrobial properties that can be used as infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

CONCLUSION

Medicinal plants continue to be an important therapeutic aid for the ailments of humankind. The search for eternal health and longevity and for remedies to relieve pain and discomfort drove early man to explore his immediate natural surroundings and led to the use of many plants, animal products, and minerals, etc. and the development of a variety of therapeutic agents. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that “green medicine” is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects.

Morinda belonging to the family of Rubiaceae, it is indigeneous to tropical countries and is considered as important traditional folk medicines. In particular, the species Morinda tinctoria is reported to have a range of therapeutic and nutritional values. There is a great demand for its leaf extract in alternative medicine for different kinds of illnesses such as arthritis, diabetes, muscle ache, menstrual difficulties, heart disease, cancer, gastric ulcer and drug addiction. In conclusion, Morinda tinctoria extracts possess a broad spectrum of activity against a panel of

bacteria responsible for the most common bacterial diseases. The potential for developing antimicrobials from higher plants appears rewarding it will lead to the development of a phytomedicine to act against microbes. Thus, the demonstration of antimicrobial activity against both bacteria is an indication that the plant is a potential source for the production of drugs. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibacterial from this plant are future challenges.

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