INTERNATIONAL JOURNAL OF CURRENT ADVANCED RESEARCH
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 11; Issue 10(A); October 2022; Page No. 1644-1647
DOI: http://dx.doi.org/10.24327/ijcar.2022.1647.0367

Review

PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL EXAMINATION OF THE ORGANIC AND AQUEOUS EXTRACT OF FRUIT OF MOMORADICA MONADELPHA (TILKOR)

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A R T I C L E I N F O

Article History:

Received 17th September, 2022
Received in revised form 27th September, 2022
Accepted 20th October, 2022
Published online 28th October, 2022

Key words:

Momoradica Monadelphа, antibacterial activity, phytochemical analysis

A B S T R A C T

The bioactive compounds of fruits of Tilkor were investigated for antibacterial activity against some pathogenic bacteria. The aqueous extracts did not show much significant activity, while the organic extracts (petroleum ether and methanol) showed the highest activity against the test bacteria. The activity was more pronounced on gram-positive organisms with Staphylococcus aureus being more susceptible and Salmonella paratyphi a being more resistant. Phytochemical analysis showed that the extracts contain alkaloids, tannins, saponins, flavonoids, glycosides and phenols.

INTRODUCTION

Most of the modern researches on herbal medicine have hinged around traditional folklore medicine. The modern medicine has brought with it an array of drugs, none of which is non-toxic and quite safer for human consumption. There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of plants for their activity is very essential and needs urgent attention in order to know the value of the plant. The screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation or ethnobotanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging long process. Importance of the plant lies in their biologically active principles. There are two types of plant chemicals, primary metabolites such as sugars, proteins, amino acids, chlorophylls, etc. The other category of chemicals is called secondary metabolites, which includes alkaloids, terpenoids, saponins and phenolic compounds. These chemicals exert a significant physiological effect on the mammalian system. Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases (Davis, 1994). The increase in resistance to microorganisms due to indiscri-minate use of antimicrobial drugs forced scientists to search for new antimicrobial substances from various sources including medicinal plants (Karaman et al., 2003). Another driving factor for the renewed interest in past 20 years has been the rapid rate of plant species extinction. Around 12,000 plant secondary metabolites of antimicrobial importance have been isolated. These compounds fall in one of the major groups of compounds like phenols, quinines, flavonoids, tannins, terpenoids, alkaloids and other mixtures (Scultes, 1978). Infectious diseases account for high proportion of health problems in the developing countries (Sashi et al., 2003). In India, about 2,500 species of plants are used for medicinal purposes and about 90% of the medicinal plants provide raw materials for the herbal pharmaceuticals, which are collected from wild habitats. The rich knowledge base of countries like India in medicinal plants and healthcare has lead to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development programs in the pursuit of discovering novel drugs (Rajasekharan and Ganeshan, 2002). However, several plants are used in India in the form of crude extracts without scientific evidence of efficacy (Ahmed et al., 1998). At this juncture it is of interest to determine the scientific basis for the traditional use of these plants. *Momoradica Monadelpha* commonly known as 'Tilkor' available in wild and cultivated form. It is the native of Central Africa, India and Asia distributed naturally in China, Tropical Asia, India, Australia and Africa. It is considered as a valuable wild vegetable by the indigenous people of Southeast Asia and India. Every part of this plant is valuable in medicine. The aim of the present study is to reveal the antimicrobial properties and phytochemicals of different extracts of Tilkor fruit through in vitro investigation.

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Table 1 Various medicinal uses of different parts of *Momoradica Monadelpha*

Leaves	When mixed with gingely oil treats ring worm, psoriasis and itch; when mixed with ghee cures sores, skin diseases, skin eruptions of small pox; causes cooling effect to eyes, heals big ulcers, small lesions of scabies, anuria and alleviate body heat.
Stem	Antispasmodic effect.
Fruit	Green fruit when chewed cures sores on tongue; raw fruit used as vegetable; dried fruit removes eczema.
Bark	Dried bark has cathartic properties
Juice	Juice of tuberous roots, stem and leaves serve as a cure for diabetes, intermittent glycosuria, enlarged glands and skin diseases such as pityriasis and also treats urinary tract infection, related troubles.
Powder	Used in treating gastro – intestinal disturbances, liver weakness, dysentery, vomiting, wormal infestation, purifies blood, curb infection in the body, effective against chronic cough and cold and gives good results for bronchitis and asthma.
Root	Tubers remove pain in joints, diabetes, skin lesions (Tenia), apthous ulcers, wheezing and phlegm.
Decoction	Decoction of stem and leaf cures bronchitis.

Table 2 Qualitative analysis of phytochemicals in Momoradica Monadelpha fruit

Phytochemical	Methanolic extract
Alkaloids	+
Steroids	+
Tannins	+
Saponins	+
Ellagic acid	+
Phenols	+
Glycosides	+
Lignans	-
Triterpenoids	+
Flavonoids	+

MATERIAL AND METHOD

Preparation of plant extracts

The plant material was collected from Medicinal plant garden of Himanshu Shekhar Malik at, Jale Darbhanga and authenticated at Department of Botany, L. N. Mithila University, Darbhanga. Coarse powder from the shade dried fruits of *Tilkor* (50 g) was extracted to exhaustion successively with petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, methanol, ethanol and aqueous extracts using a soxhlet apparatus. These extracts thus obtained were dried under reduced pressure at room temperature not exceeding 40°C and were used for the assays. The solvents used were purchased from the instrument, Darbhanga Tower and all the solvents were of analytical grade.

Phytochemical screening

Chemical tests were carried out on the methanol extract to identify the constituents utilizing standard methods of analysis (Gibbs, 1974; Trease and Evans, 1989).

Bacterial cultures

Six bacteria, two gram positive, *Staphylococcus aureus*, *Bacillus cereus* and four gram negative, *Pseudomonas putida*, *Salmonella paratyphi A*, *S. paratyphi B* and *Klebsiella pneumoniae* were used for the bioassay. The pure strains were obtained from Microbial Type Culture Collection from D.M.C.H. Darbhanga. The organisms were maintained on agar slopes at 4°C and subcultured for 24 h before use.

Bacterial susceptibility testing

The agar plate well diffusion method was used as described by Desta (2005). A standardized inoculum 1 to 2 x 107 cfu/ml 0.7 MC Farland standards was introduced onto the surface of sterile agar plate and evenly distributed the inoculum by using a sterile glass spreader. Simultaneously 8 mm wells were cut from the plate using a sterile cork borer. 200 l of extract at a concentration of 200 mg/ml was introduced into each well. The agar plates were incubated aerobically at 37°C. After 24 h the inhibition zones were measured with a ruler and compared with the control well containing only solvent and 10 mg/ml of streptomycin served as control.

Statistical analysis

All the tests were conducted in triplicates. The data of all the parameters were statistically analysed and expressed as mean ± S.D.

Table 3 Antibacterial activity of different solvent extracts of *Coccinia indica* fruit by well diffusion method (Zone of inhibition in mm)

Solvent (100 g/ml)	Gram positive		Gram negative			
	Staphylococcus aureus	*Bacillus cereus*	*Pseudomonas sputida*	*Salmonella paratyphi A*	*Salmonella paratyphi B*	*Klebsiella pneumoniae*
Petroleum ether	19 ± 0.3	12±0.3	17±0.2	9±0.2	8±0.3	15±0.3
Diethyl ether	11± 0.2	10±0.3	13±0.2	1±0.2	1±0.3	9±0.3
Chloroform	7 ± 0.3	1±0.3	1±0.3	-	2±0.2	4±0.2
Ethyl acetate	11 ± 0.3	10±0.2	5. ±0.2	-	1±0.2	9±0.2
Acetone	10 ± 0.2	18±0.3	1±0.2	-	-	6±0.3
Methanol	8 ±0.2	15±0.2	13±0.2	3±0.2	9±0.3	8±0.2
Ethanol	11±0.2	10±0.3	11±0.2	-	-	7+0.2
Aqueous	13±0.2	6±0.2	2±0.3	6±0.2	1±0.2	3±0.2
Streptomycin (10 mg/ml)	16±0.3	14±0.1	15±0.2	10±0.2	12±0.3	13±0.

The profile of uses of different parts of Tilkor is shown in Table 1. Phytochemical screening was carried out on the methanolic extract which revealed the presence of alkaloids, steroids, tannins, saponins, ellagic acids, phenols, glycosides and triterpenoids. Lignans were absent (Table 2). Some of the phytochemicals reported are known to possess various biological activities. Oral administration of water soluble alkaloid fraction reduced fasting blood glucose of guinea pig by 29.3 and 21% in OGTT (1 gm/kg) conducted on rats (Mukherjee et al., 1972). Triterpenoids like taraxerone have exhibited anti-inflammatory analgesic and hepatoprotective activity (Rao et al., 2003). Feeding of water soluble alkaloid fraction (1 gm/kg) of C. indica leaves to normal fasting guinea pigs showed hypoglycaemic activity of short duration and the effect was due to the presence of betasitosterol, a phytosterol (Mukherjee et al., 1972). Proteins like glucokenin have the property of reducing the amount of sugar in the blood (Sambasivam, 1931; Murugesa, 1988; Nadkarni, 2000).

Eight different solvent extracts of Tilkor fruit were tested against six gram negative and gram positive bacteria. Results of antibacterial activity of all the extracts and their efficacies as compared to standard and depicted are shown in Table 3. The crude extracts of Tilkor exhibited moderate to significant antibacterial activity against all tested bacteria with inhibition zone ranging from 1 - 19 mm and comparable to standard. Petroleum ether extract was the most active and showed considerable antibacterial activity against all tested gram positive and gram negative bacteria producing a maximum inhibition zone of 19 mm against S. aureus. Other tested extracts also inhibited the growth of a number oftest organisms but to a lesser extent and were mainly active against the gram-positive S. aureus. The relatively high anti-staphylococcal activity might, however, be of interest since several Staphylococcus strains are reported to express a multi drug resistance and natural plant extracts were shown to have good anti-staphylococcal activity (Lechner et al., 2004). The study also revealed that methanol extract was found to be active against B. cereus and P. putida producing inhibition zone 15 and 13 mm, respectively. None of the extracts showed significant antibacterial activity against S. paratyphi A and B. So these bacterial strains are considered most resistant towards all the extracts tested. Least activity was shown by chloroform extract against all the tested bacteria with inhibition zone ranging from 1 - 7 mm.

The present study revealed the moderate activity of water extract agreeing with earlier reports that use of organic solvents is always better for extraction and isolation of antibacterial compounds (Varadarajan et al., 2007). In addition, the effectiveness of the extracts was not due to one main constituent, but to the combined action of other chemical compounds involved in it (Bai, 1990). Some examples include alkaloids, flavonoids, terpenoids, thymol and other compounds of phenolic nature which are classified as antimicrobial compounds (Rojas et al., 1992). These findings on antibacterial activity support the claim of the traditional healers that Tilkor has been used to relieve pneumonia, dysentery, cough and cold.

Even though the present study on these extracts is an addition to the scientific literature, detailed investigations are needed to isolate bioactive principles from these extracts.

ACKNOWLEDGMENT

Authors were thankful to Prof. Shashi Shekhar N. Sinha (Retd. Univ. Prof. & Head University, Dept. of Botany, B.R.A. Bihar University, Ex. Director Rajendra Agriculture University, Pusa and Eminent International Scientist of Radiation Genetics) for his guide lines and Darbhanga Medical College, Darbhanga as well as Dr. Rajendra Prasad Central University Pusa for providing their technical support in this project.

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How to cite this article:
Panshu Pratik and Shabina Perween., (2022). Phytochemical investigation and antimicrobial examination of the organic and aqueous extract of fruit of momoradica monadelpha (Tilkor). *International Journal of Current Advanced Research*, 11(10), pp.1644-1647. DOI: http://dx.doi.org/10.24327/ijcar.2022.1647.0367

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