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Research Article

Antimicrobial Activity of Formulation Containing Bioactive Extract of Endocarp *of Randia spinosa*

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ABSTRACT

The antimicrobial activity of Randia *spinosa (Family* Rubiaceae) *was* carried out using cup-plate method by measuring zones of inhibition. The gel was formulated using suitable base and subjected to antimicrobial activity. The organisms used were *Staphylococcus aureus, Streptococcus mutans, Aspergillus niger and Candida albicans.* The standard drugs used were Ciprofloxacin **(100µg/ml) and Amphotericin B (100µg/ml) for** bacteria and fungi respectively. The gel showed significant activity against these pathogens when compared to the standard drug (placentrex gel 10%). The gel base does not show any antimicrobial activity.

Keywords: *Randia spinosa,* Anti-bacterial, anti-fungal, cup-plate method.

INTRODUCTION

Randia spinosa (Poir.) Rubiaceae is a deciduous, thorny shrub.[1] The various parts of the plant (leaves, roots, seeds and fruits) are widely used by various tribal communities and forest dwellers for the treatment of variety of ailments. The fruits of the plant are claimed to posses' medicinal virtues as it is used as emetic and antipyretic. The plant is also documented to posse's beneficial effects as antinociceptive, anthelmintic, antispasmodic, anticancer, anti-inflammatory, immunostimulant, antileishmanial, antitumor, astringent, and diaphoretic. [2, 3, 4]. It is believed to be useful in bronchitis, asthma, leucoderma, and diseases of the brain. Following various folk claims for cure of numerous diseases, efforts have been made by researchers to verify the efficacy of the plant through scientific biological screenings.[5]

Tandon et al., showed antileishmanial activity, Baghdikian et al., and Recio et al., displayed anti-inflammatory activity, Abdel-Kader et al., showed antitumor activity of various extract of R. spinosa. [6- 8.] The ethanolic extract of the pulp showed a stimulant action on isolated guinea pig uterus. In experimental animals, crude saponins produced salivation; on contact it caused a generalized irritation of the mucous membranes producing sneezing, vomiting, and bleeding from the urinary tract.

In a study, anti-inflammatory activity [8] on alcohol and aqueous extract of R. spinosa was evaluated using carrageenan induced rat paw oedema in albino rats. The extract treated group of rats showed significant reduction in paw volume when compared to reference standard indomethacin treated group of rats. The air dried powdered fruits extracted with a mixture of dichloromethane and methanol (1:1%, v/v) under reflux when evaluated for antibacterial and antifungal activity by agar dilution-streak method, growth of Saccharomyces cerevisiae were observed after 4 days [9, 10]. In current scenario, ethno botanical and traditional uses of plant originated compounds has gained much attention as they are well tested for their efficacy and generally believed to be safe for human use. Following the traditional and folk claims, very little efforts have been made by the researchers to explore the therapeutic potential of this plant.[11,12] An iridoid glucoside: randinoside, along with five known iridoids: galioside, deacetylasperulosidic acid methyl ester, scandosidemethyl ester, geniposide and gardenoside, were isolated from the stems of Randia spinosa.[13]

Plant

MATERIALS AND METHODS

Randia spinosa

*Randia spinosa (*Poir.) Rubiaceae is a deciduous, thorny shrub or a small tree, up to 9m in height and 90 cm in girth with a bole 2-3 m found throughout India, up to an elevation of 1350 m. in the hills**.** The plant is found in Brazil, India, Malaya, China, Ceylon, Sumatra, and East tropical Africa. Fruit is like a small crab apple, yellowish, broadly ovoid, smooth or obscurely longitudinally ribbed, crowned with the large calyx-limb, 2-celled, glabrous; pericarp thick.

Chemical Constituents

The phytochemical studies on the fruits of R. spinosa revealed presence of mixture of saponins .The saponins occur in the fruit at all stage of ripening[14]. The fruits of R. spinosa contain toxic saponins of oleanolicacid. They also contain leucocyanidin and mannitol. The saponins are concentrated mostly in the pulp [15, 16]. A mixture of two saponins, viz. randialic or neutral saponin and randialic acid or acid saponins has been isolated from the pulp[17]. On complete hydrolysis both the saponins yield Oleanolic acid as Sapogenin. Ursosaponin, isolated from the ethanolic extract of the dried whole fruit, gave ursolic acid and glucose. Randianin, isolated from the fruit, gave a haemolytic triterpenoid saponins.

Traditional uses

Most of the parts of Mainphal are of medicinal importance and used traditionally for the treatment of various ailments[1, 3, 5, 6]. The roots of the plant are considered as insecticidal and insect repellent. The seeds of the plant are used as tonic to induce appetite. The bark is astringent and is given in diarrhoea and dysentery. An infusion of the bark is used as an emetic. It is also reported to be abortifacient. As per Ayurvedic claim, Mainphal is bitter, aphrodisiac, emetic, antipyretic, carminative, cures abscesses, ulcers, inflammations, tumours, skin-diseases etc. The fruits of the R. spinosa are most popular and considered as good remedy in skin diseases, gastrointestinal tract diseases, wounds etc.

In Ceylon, the root decoction is taken for diarrhoea and biliousness. In Indo-China the powdered fruit is used as an emetic; the pounded root is employed to kill fish. The seeds are said to be used as tonic to induce appetite. The fruit in combination with other drugs is prescribed for the treatment of snake- bite and scorpion sting. The fruit is one of the ingredients of the Tanjore Pill, A famous snake remedy.

EXPERIMENTAL WORK

The fruits of *Randia spinosa* belong to the family Rubiacae were collected, identified and authenticated from botany department, Govt.College, University Campus, Nagpur. The endocarp of fruits was removed, dried and subjected to extraction. The coarsely powdered endocarp of fruit was extracted with petroleum ether using maceration technique for one week. The extract was filtered, evaporated.

It was then dried and used for further studies. The antimicrobial activity of *Randia spinosa was* carried out using cup plate method by measuring zones of inhibition. The gel was formulated using Carbopol 934 (1.5%) and subjected to antimicrobial activity[18]. The organisms used were *Staphylococcus aureus, Streptococcus mutans, Aspergillus niger and Candida albicans.* The standard drugs used were Ciprofloxacin (100µg/ml) and Amphotericin B (100µg/ml) for bacteria and fungi respectively. In present study in-vitro evaluation of antimicrobial activity of extract and gel formulation containing extract was done by Agar diffusion (cup plate) method [19].

Evaluation of Antimicrobial activity

The evaluation of antimicrobial activity of petroleum ether extract of endocarp of *Randia spinosa* was done through *in vitro* methods by agar diffusion technique. Antibacterial studies were carried out against *Staphylococcus aureus (MTCC 3160) and Streptococcus mutans (MTCC 890)* using nutrient agar medium. Antifungal studies were carried out against *Candida albicans (NCIM 3100) and, Aspergillus niger (MTCC 281) using* sabouraud dextrose agar.

Sample preparation

Stock solution

The extract was emulsified in suitable quantity of DMSO to get 5.0 % solution. (1.0 % previously sterilized Tween 80 solution is added)

Agar diffusion method

Principle

The agar diffusion method depends upon diffusion of antibiotic from a vertical cylinder through a solidified agar layer in a Petri plate to an extent such that the growth of the added micro organisms is prevented entirely in a zone around the cylinder containing a solution of the antibiotic [20].

Method

Add 0.1 ml of the inocculum/10 ml of previously molten nutrient agar media, shake well to disperse equally and immediately pour in sterile plates, allow solidifying taking care that the thickness of layer is uniform. Allow to diffuse at a temperature between 4 to 8°C and then incubate for 24 hours at37°C.Measure the zone of inhibition using a Vernier scale.

Estimation of antifungal activity by agar diffusion method

Method

Add 0. 1 ml of the inocculum/10 ml of previously molten sabouraud dextrose agar media, shake well to disperse equally and immediately pour in sterile plates, allow to solidify taking care that the thickness of layer is uniform. Allow to diffuse at a temp between 4 to 8°C and then incubate for 72 hours at22-27°C.Measure the zone of inhibition using a Vernier scale.

RESULTS AND DISCUSSION

The petroleum ether extract of endocarp showed significant antimicrobial activity against bacteria & fungi as compared to standard drug. The gel showed significant activity against pathogens when compared to the standard drug (placentrex gel 10%). (Table and Graph). The gel base does not show any antimicrobial activity. *Phyto*chemical review shows the presence of saponins in extract. The activity justifies the use of *Randia spinosa* in various infectious diseases. The antibacterial and antifungal activity of the extract may be due to the presence of saponins as revealed by phytochemical analysis.

CONCLUSION

The extract prepared in petroleum ether was evaluated for *Staphylococcus aureus, Streptococcus mutans; Aspergillus niger* and *Candida albicans* concluded that the crude extract of Randia spinosa exhibited significant antimicrobial activity and properties that support folkloric use in the treatment of some diseases as broad spectrum antimicrobial agents. The activity of the drug may be attributed to the presence of saponins which occur to the extent of 2-3 % in fresh fruits and about 10 % in dried whole fruit [14].

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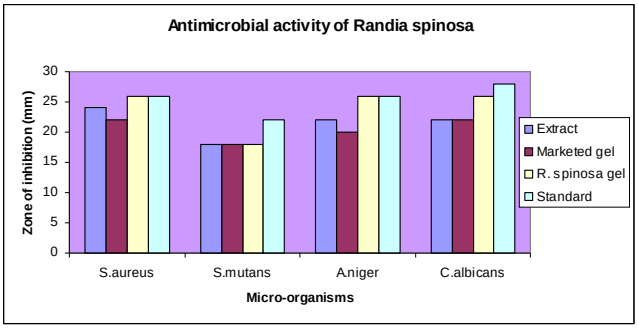
Table: Antimicrobial activity of extract of *Randia spinosa*

S. No	Organism	Zone of inhibition in mm						
		Petroleum ether extract			Formulation		Ciprofloxacin	Amphotericin B
		A	b	C	A Marketed Gel	B Randia spinosa gel		
1	Staphylococcus aureus	20	20	24	18	22	22	
2	Streptococcus mutans	18	22	22	18	20	22	
3	Aspergillus niger	16	20	26	18	26		26
4	Candida albicans	16	24	26	22	26		28



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