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.
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. The fruits of R. spinosa contain toxic saponins of oleanolic acid. They also contain leucocyanidin and mannitol. The saponins are concentrated mostly in the pulp. A mixture of two saponins, viz. randialic acid or acid saponins and randialic acid or acid saponins has been isolated from the pulp. 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. 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 Rubiaceae 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. The organisms used were Staphylococcus aureus, Streptococcus mutans, Aspergillus niger and Candida albicans. The standard drugs used were Ciprofloxacine (100μg/ml) and Amphterocin 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.

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. (10 % previously sterilised 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.

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 at 37°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. Phytochemical 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

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