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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF SAPINDUS MUKOROSSI

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ABSTRACT

Sapindus mukorossi Geartn, is an extremely valuable medicinal plant distributed in tropical and subtropical region of Asia. Plant extracts appear to be one of the better alternatives as they known to have minimal environmental impact and danger to consume in contrast to synthetic pesticide. The phytochemical screening of methanol extract releaved the presence of various secondary metabolise such as alkaloids, amino acids, carbohydrates, volatile oil, saponins, tannins, sterols and proteins. The antimicrobial activity was done for ethanol extract. The study revealed that the ethanolic extract of the pericarp of *sapindus mukorossi* showed more prominent antibacterial activity against *E.coli, staphylococcus aureus* than the aqueous extract.

KEYWORDS: sapindus mukorossi, sapindaceae, phytochemical, antimicrobial.

INTRODUCTION

Sapindus mukorrosi Geartn, a member of the family Sapindaceae, is commonly known by several names such as soapnut, soapberry, washnut, reetha dodan and doadni. It is a deciduous tree widely grown in upper reaches of Indo-Gangetic plains, shivaliks and sub Himalayan tracts at altitudes from 200 m to 1500 m. The *Sapindus mukorossi* is a fairly large, deciduous tree with a straight trunk up to 12 m in height, sometime attaining a height of 20 m and a girth of 1.8 m, with a globose crown and rather fine leathery foliage. Bark is dark to pale yellow, fairly smooth, with many vertical lines of lenticels and fine fissures exfoliating in irregular wood scales. The blaze is 0.8 to 1.3 cm, hard, not fibrous, pale orange brown, brittle and granular. Leaves are 30 to 50 cm long, alternate, paripinnate; common petiole very narrowly bordered, glabrous; leaflets 5-10 pairs, opposite or alternate, 5-18 by 2.5-5 cm,

lanceolate, acuminate, entire, glabrous, often slightly falcate or oblique; petioles 2-5 m long. Inflorescence is a compound terminal panicle, 30 cm or more in length, with pubescent branches. Flowers are about 5 mm across, small, terminal, polygamous, greenish white, subsessile, numerous, mostly bisexual. Sepals 5, each with a woolly scale on either side above the claw. Fruits are globose fleshy, 1-seeded drupe, sometimes two druples together, about 1.8 to 2.5 cm across. Seeds are 0.8 to 1.3 cm in diameter, globoose, smooth, black and loosely placed in dry fruit.

The fruit is valued for the saponins (10.1%) present in the pericarp and constitutes up to 56.5% of the drupe known for inhibiting tumor cell growth. In China and Japan it has been used as a remedy for centuries. In Japan its pericarp is called “enmei-hi”, which means “life prolonging pericarp” and in China “wu-huan-zi”, the “non-illness fruit”. The major compounds isolated from *sapindus mukorossi* are tritepenoidal saponins of mainly three oleanane, dammarane and tirucullame types. Recently many of the pharmacological action of this plant have been explored which include the antimicrobial[19], cytotoxic, molluscicidal insecticidal, piscicidal and fungicidal, activities. One of the talked about activities of this plant is the contraceptive activity of the saponins extracted from the pericarp of the fruit.

ANTIMICROBAL ACTIVITY

Microbiological assay may be defined as qualitative or quantitative determination of any chemical compound from a simple or even complex material with the use of microorganisms. It is necessary to assay antimicrobial agents for determination of potency, for determining the pharmacokinetics of a drug in animal or man and for monitoring and controlling antimicrobial chemotherapy. Quantitative chemical or physical methods can assay most of the currently employed therapeutic agents. Many therapeutic agents, which either inhibit the growth of microorganisms (antibiotics) or are essential for their growth (vitamins and amino acids) can be standardized by microbiological assays.

Microbiological assays are relatively as accurate as chemical methods. It is simple, specific, inexpensive and convenient method. Compared with biological assay methods using animals, microbiological techniques possess the advantages of minimal requirements of space, labour, material and time, microbiological assays are very useful for detecting changes in potency of antibiotics and their preparation, microbial assays are more difficult to perform as compared to physical and chemical assays and also require proper calibration, they are reproducible and

have greater error as compared to other assays. Microbiological assays are not used if a good alternative physical or chemical assays are available.

Assay method

The microbiological assay of antibiotic may be carried out by the following method:

Cup plate or cylinder plate method

This method depends on the diffusion of an antibiotic from a vertical cavity or a cylinder, through the solidified agar layer in a perti plate. The growth of test microorganism is inhibited entirely in a circular area or zone around the cavity or cylinder containing a solution of the antibiotic.

A liquefied assay medium (43 to 45^0) is inoculated by suspension of test microorganisms and the inoculated medium is poured immediately into sterile perti plate or pre-prepared agar plates by using assay medium and then spread the test culture or microorganisms on the surface of plates of plates.

Solution of known concentration of the standard preparation and the test antibiotic are prepared in appropriate solution. Preparation of the standard solution and potency of antibiotics for assay of penicillin and assay of streptomycin. These solutions are added in sterile cavities or cylinders prepared in solid medium.

The volume of solution assed to each cavity or cylinder must be uniform the hopes. When paper discs are used, them and sufficient to fill these disc should be sterilize first and then dipped in the standard solution or the solutions and placed on the surface of the medium.

The plates are left standing 1 to 2 hours at room temperature or at 4^0 as a period of pre-incubation diffusion to minimize the effects of variation in time between the application of the different solution. All plates are then incubation for about 18 to 24 hours at the temperature incubated. Accurately measure the diameters or areas of the circular inhibition zones produced by standard and test antibiotic solution. Plot the graph which relates zones diameter to the logarithm of the concentration of antibiotic and calculate of test antibiotics.

MATERIALS AND METHOD

SL No.	CHEMICALS/REAGENTS	MANUFACTURED BY
01.	Nutrient agar	Himedia laboratories pvt Ltd.
02.	Agar agar	Himedia laboratories pvt Ltd.
03.	Peptone	Himedia laboratories pvt Ltd.
04.	Methanol	Sd fine limited.
05.	Amikacine injection	Troika pharmaceuticals Ltd.
06.	Ninhydrin reagent	Nice chemicals pvt Ltd.
07.	Dragendroffo's reagent	Pallav chemicals and solvents pvt Ltd.
08.	Mayer's reagent	Karnataka fine chemicals.
09.	Molish's reagent	Nice chemicals pvt Ltd.
10.	Sudan III reagent	Himedia laboratories pvt Ltd.
11.	Barfoied reagent	Nice chemicals pvt Ltd.
12.	Biyuret reagent	Finar chemicals limited.
13.	Sulphuric acid	
14.	Ferric chloride	Finar chemicals limited.
15.	Distilled water	

SL No.	APPARATUS USED	MANUFACTURED BY
01.	Soxhlet apparatus	Borosilicate glass
02.	Round bottom flask	Borosilicate glass
03.	Beakers	Borosilicate glass
04.	Conical flask	Borosilicate glass
05.	Test tubes	Borosilicate glass
06.	Test tube holder	Iron
07.	Heating mantle	Bio-techniques India
08.	Weighing balance	Electronic precision balance
09.	Petry plates	Borosilicate glass
10.	Measuring cylinder	Borosilicate glass
11.	Spatula	Stainless steel
12.	Glass rod	Borosilicate glass
13.	Funnel	Borosilicate glass
14.	Wattman filter paper	
15.	Butter paper	

SL.No	Ingredients	Formula
01.	Nutrient agar	3 gm
02.	Agar agar	15 gm
03.	Peptone	5 gm
04.	Methanol	300 ml
05.	Amikacine Injection	2 gm
06.	Distilled water	1000 ml

DISC DIFFUSION METHOD

When a filter paper disc impregnated with a chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size

of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "zone of inhibition".

Antiseptics, disinfectants and antibiotics are used in different ways to combat microbial growth. Antiseptics are used on living tissue to remove pathogens. Disinfectants are similar in use but are used on inanimate objects. Antibiotics are substances produced by living organisms, such as *Penicillium* or *Bacillus*, that kill or inhibit the growth of other organisms, primarily bacteria. Many antibiotics are chemically altered to reduce toxicity, increase solubility, or give them some other desirable characteristic that they lack in their natural form. Other substances have been developed from plants or dyes and are used like antibiotics. A better term for these substances is antimicrobials, but the term antibiotic is widely used to mean all types of antimicrobial chemotherapy. Many conditions can affect a disc diffusion susceptibility test. When performing these tests certain things are held constant so only the size of the zone of inhibition is variable. Conditions that must be constant from test to test include the agar used, the amount of organism used, the concentration of chemical used, and incubation conditions (time, temperature and atmosphere).

The amount of organism used is standardized using a turbidity standard. This may be a visual approximation using a McFarland standard 0.5 or turbidity may be determined by using a spectrophotometer (optical density of 1.0 at 600 nm). For antibiotic susceptibility testing the antibiotic concentrations are predetermined and commercially available. Each test method has a prescribed media to be used and incubation is to be at 35-37o C in ambient air for 18-24 hours.

The disc diffusion method for antibiotic susceptibility testing is the Kirby-Bauer method. The agar used is Meuller-Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disc diffusion method. This method is well documented and standard zones of inhibition have been etermined for susceptible and resistant values. There is also a zone of intermediate resistance indicating that some inhibition occurs using this antimicrobial but it may not be sufficient inhibition to eradicate the organism from the body.

The standardized methods for antiseptic and disinfectant testing are more rigorous and more difficult to reproduce in a student laboratory. Two common tests are the Phenol Coefficient

Test (a comparison of the effect of the chemical and phenol on several organisms) and the Use Dilution Test (testing the chemical under actual conditions of use). A disc diffusion test can be used to approximate the Use Dilution Test. The chemical under consideration is used to saturate a filter paper disc. This disc is then used to introduce the chemical to the agar for testing. The actual zone sizes have not been standardized as in the Kirby-Bauer method, but a comparison of zone sizes for the same chemical among organisms will provide a n approximate effectiveness of the chemical.

RESULTS

SL NO.	Micro organisms	Zone of inhibition (mm) Methanol extract (µg/ml)			
		100µg/ml	200µg/ml	300µg/ml	350µg/ml
01.	S. Aureus	5mm	NA	6mm	5mm
02.	E. Coli	5mm	NA	NA	NA

S.aureus

E.coli

DISCUSSION

After screening of phytochemicals with various chemical tests gives alkaloids, amino acids, carbohydrates, volatile oils, saponins, tannins, steroids and proteins. The various results then again determination of zone of inhibition with different concentration of plant extract here maximum concentration is consider for zone of inhibition compared with standard drug solution.

CONCLUSSION

The present study used to evaluate the various concentrations of plant extract and measure the zone of inhibition of various concentrations of samples at the same time finding the various chemicals like alkaloids, amino acids, carbohydrates, volatile oils, saponins, tannins, steroids,

proteins. The concentration of 100µg, 200 µg, 300µg, 350µg the zone of inhibition at 300µg is 6 mm it is better than other.

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