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Phytochemical screening and antimicrobial activity of *Strychnos potatorum* L.f (root bark)

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ABSTRACT

The present research work attempt to evaluate the antibacterial activity and also to screen phytochemicals present in the *Strychnos potatorum* L.f. Disk diffusion method was used to study the antibacterial activity against *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginos, Enterococci faecalis, Klebsiella pneumonia* and *Proteus mirabilis.* In this study, we observed that the samples in different solvent extract had antibacterial activities by formation of inhibitory zone. Qualitative phytochemical analysis of samples in different solvent extract confirms the presence of various phytochemicals like Carbohydrates, Saponins, Tannins, Flavonoid, Alkaloids, Glycosides, Proteins, Phytosterol & Steroids, Phenols, and Terpenoids. The solvent Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate and aqueous extract gave good results. In this study we concluded that different solvent extract of *Strychnos potatorum* L. f showed good antibacterial activity and it may be attributed to the presence of phytochemicals and may be used as antimicrobial agents.

Keywords: Antibacterial activity, Qualitative phytochemical analysis, *Strychnos potatorum* L.f, zone of inhibition.

INTRODUCTION

Man and nature are in interrelationship from ages far back. The curative aid derieved from Nature has mark golden mark between man and environment. Plant medicines are nature's gift, which has bestowed its blessings to cure many diseases. Plants contain many chemical compounds of therapeutic use which are known as phytochemicals. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans

(Hasler and Blumberg, 1999). Secondary plant metabolites are the basis of treatment of many microbial diseases in humans. Plant medicine are culturally acceptability because of better compatibility and adaptability with the human body and also these medicines pose lesser side effects. Hence in recent years plant medicines are being used in treatment of various incurable microbial diseases. In some cases, screening tests have been performed for extracts in different solvents, which gives preliminary information about the content of various classes of compounds in seaweed (Whankatte and Ambhore 2016).

Strychnos potatorum L.f belong to family Loganiaceae. A medium sized glabrous tree of hight 11to 13 m. Stem is fluted, bark cracked and scaly with very deep and narrow vertical and thin ridges which easily break off. Branches are swollen at nodes. Leaves 2-3 by 1-1.7 inch.,sessile, subcoriaceous, ovate or elliptic, acute, glabrous and shining, spuriously 3 or 5 nerved. The lateral nerves spinging from the lower part of the midrib not far from its base, The base are rounded or acute. Petioles 2-2.5 mm long. Flowers rather large for the genus, in short almost glabrous nearly sessile axillary cyme; pedicels so short. Calyx 2mm long glabrous, segments 5, ovate, acute. Corolla 5 lobed , lobes are 2.5 mm long, with a tuft of hair inside towards the base of each lobes. Ovary ovoid, glabrous, tapering in to a long glabrous style. Stigma obscurely two lobed. Fruit is berry, black when ripe, Seeds 1-2 circular, globose in shape and not greatly compressed, whitish, shining with short yellow silky hairs.

MATERIALS AND METHODS

Plant material

The root bark of plant *Strychnos potatorum* L.f was collected in fresh condition from Salbardi Multai District, Madhya Pradesh, India. The plant was authenticated by well known taxonomist Dr. S. M. Bhuskute Principal, Bhavbhuti Mahavidyalaya, Amgaon, district Gondia, Maharashtra. Voucher specimen has been deposited at Bharatiya Mahavidyalaya, Amravati, Maharashtra. The root bark washed under running water and dried under shade then ground into a fine powder using blender and stored in plastic bottle at room temperature.

Preparation of extracts

The extraction of soluble compounds from *Strychnos potatorum* L.f by the Soxhlet method was performed using Petroleum ether, Benzene, Acetone, Chloroform and Ethyl acetate and Distilled water as solvents. 25 gm of dried powdered plant sample were taken in a Whatman No.1 filter paper cone and placed into Soxhlet apparatus. 100 ml of above solvents were taken successively, in the round bottom flask attached to the Soxhlet apparatus. A condenser was attached to this setup. Then the whole setup was placed on a heating mantle. The temperature was set in the range of 25-30^{0}C. solvents gets vaporized and rises up to the condenser where it condenses back into liquid. This liquid falls into the plant sample in the cone and extracts certain compounds and falls back into the round bottom flask. This process was continued till all the compounds get extracted from the plant. The extracts obtained from the above process was evaporated and stored in cap glass vials.

Phytochemical analysis

Preliminary qualitative phytochemical screening of samples was carried out with the following methods describe by Harborne (1973).

Test for Detection of carbohydrates

Molisch's Test: Small Quantity of Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate, Distil water extract were taken separately in 10 ml of distil water and two drops of Ethanolic naphthol (20%) and 2ml of concentrated Sulphuric acid were added, formation of reddish violet ring at the junction indicates presence of carbohydrates.

Test for Detection of Saponins

Foam Test: 2 ml of Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate, Distil water extract were taken and added two equal amount of distil water and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins (Kumar *et al*., 2009).

Test for Detection of Tannins

Ferric Chloride Test: Small Quantity of Petroleum ether, Benzene, Acetone Chloroform, Ethyl acetate, Distil water extract were taken separately in water, 2-3 drops of 5%

ferric chloride was added. Formation of black or green colour indicates the presence of tannins.

Test for Detection of Flavonoids

Sulphuric Acid Test: A fraction of extract was treated with concentrated sulphuric acid and observed for formation of orange colour.

Test for Detection of Alkaloids

Mayer's Test: To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added then few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for Detection of Glycosides

Sulphuric Acid Test: To 2ml of plant extract, 1ml of glacial acetic acid and 5% ferric chloride was added then few drops of concentrated sulphuric acid were added. Presence of greenish blue colour indicates the presence of glycosides.

Test for Detection of Proteins and Amino acids

Ninhydrin Test: To 2ml of plant extract, few drops of 0.2% Ninhydrin was added and heated for five minutes. Formation of blue colour indicates presence of proteins.

Test for Detection of Steroids and phytosterols

Sulphuric Acid Test: To 1 ml of plant extract, equal volume of chloroform and few drops of concentrated sulphuric acid were added. Formation of brown ring indicates the presence of steroids and formation of bluish green colour indicates the presence of phytosterols.

Test for Detection of Phenols

Ferric Chloride Test: To1 ml of plant extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green colour indicates presence of phenols.

Test for Detection of Terpenoids

Salkowski test: 2ml of plant extract was mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. Reddish brown coloration of the interface is formed indicating the presence of terpenoids.

Antimicrobial Activity:

Recent era can be said to the the era of Pandoras box which is full of microbes that become susceptible to antibiotics. The life span of any antibiotic is limited and so the public is becoming increasingly aware of problems with the overprescription and misuse of synthetic drugs. Plants have an almost limitless ability to cure all types of diseases hence the role of medicinal plants is very important in treatment of various diseases in human being. Medicinal plants are the first home remedy that a person relies on for infections. Higher plants have shown to be potential source of new antimicrobial agent (Mitscer, 1987). It is traditionally-held belief that the synergistic combination of several active principles in plant preparations is responsible for their beneficial effects.The newly emerging Viral and bacterial diseases are challenge before modern medicine, hence scientist are showing great interest for the discovery of new effective plant based drugs for treatment of these disorders.

Collection of Bacterial and Parasitic Isolates

Bacterial isolates of *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginos, Enterococci faecalis, Klebsiella pneumonia* and *Proteus mirabilis* were obtained from Samruddhi Microbiology Diagnostic Laboratory, Amravati which is run by Dr. S. R. Gulhane (Microbiologist). The isolates were authenticated by biochemical tests as described by Cheesebrough (1985) preserved on potato dextrose agar and nutrient agar respectively and stored at 4^0C until ready to use.

Disc Diffusion Method

The bacterial isolates of *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginos, Enterococci faecalis, Klebsiella pneumonia* and *Proteus mirabilis* were subculture overnight at 37^0C on potato dextrose agar and nutrient agar plates respectively. Six plate per organism. The suspension of each bacterial and parasitic isolates were prepare as described by John *et al.* (1999) in isotonic sodium chloride solution. Solidified petridishes, for each microorganism for six solvents on Muller- Hinton agar were flooded with the appropriate suspension of bacterial isolates respectively.

Sterile 6 mm diameter absorbent filter papers disc (punched out from No.1 Whatman paper) were impregnated with respective solvents of plant extracts namely Petroleum ether, Benzene, Acetone, Chloroform,

Ethyl acetate, Distil water extracts and placed on inoculated lawn. Six extracts from each plant parts in ten plants were tested for antimicrobial sensitivity. All the plates were kept for incubation period i.e. 24 hrs. for bacteria. Results were noted down in terms of sensitivity zone around the disc which is measured in millimeter (mm) and results were sequentially recorded in the tabular form.

RESULTS AND DISCUSSION

Table:- 1 represents various photochemical present in different extracts. The *Strychnos potatorum* L.f extract of root bark in petroleum ether shows presence of carbohydrates, alkaloids and phytosterols. While the extract in benzene shows the presence of carbohydrates, flavonoids, alkaloids, phytosterols & steroids and terpenoids. Acetone extract depicts the presence of carbohydrate, flavonoids, alkaloids, glycosides, phytosterols and terpenoids. The presence of carbohydrates, saponins, flavonoids, alkaloids, glycosides, phytosterols & steroids and terpenoids was determined in chloroform extract. The ethyl acetate shows the presence of carbohydrates, saponins, flavonoids, alkaloids, glycosides, phytosterols and terpenoids. Significant amount of carbohydrates, saponins,tannins, flavonoids, alkaloids, phytosterols & steroids and terpenoids were present in aqueous extract of *Strychnos potatorum* L.f (root bark).

Table 1: Preliminary phytochemical screening of *Strychnos potatorum* L.f

Sr. No	Secondary Metabolite	B1 Petroleum ether	B2 Benzene	B3 Acetone	B4 Chloroform	B5 Ethyl acetate	B6 Distil water
1	Carbohydrates	++	+	+	++	++	++
2	Saponins	---	---	---	+	---	+
3	Tannins	---	---	---	---	---	+
4	Flavonoids	---	+	+++	+	+++	+++
5	Alkaloids (Mayer's test)	+	+++	+	++	+	+
6	Glycosides	---	---	+	+	++	---
7	Proteins	---	---	---	---	---	---
8	Phytosterol	---	---	---	---	---	---
9	Steroids	++	+	++	++	+++	++
10	Phenols	---	---	---	---	---	---
11	Terpenoide	---	+	++	++	+++	+++

Table 2: Antimicrobial activity of *Strychnos potatorum* L.f (root bark)

Sr,no	Micro-organism	Petroleum ether	Benzene	Acetone	Chloroform	Ethyl acetate	Distiled Water
1	*Staphylococcus aureus*	00	00	00	**10 mm**	00	00
2	*Enterococcus faecalis*	00	**10 mm**	**11 mm**	**13 mm**	**10 mm**	00
3	*Escherichia coli*	00	00	00	00	00	00
4	*Pseudomonas aeruginosa*	00	00	00	00	00	00
5	*Klebsiellia pneumoniae*	00	00	00	**11mm**	00	00
6	*Proteus vulgaris*	00	**11 mm**	00	**12 mm**	**10 mm**	00

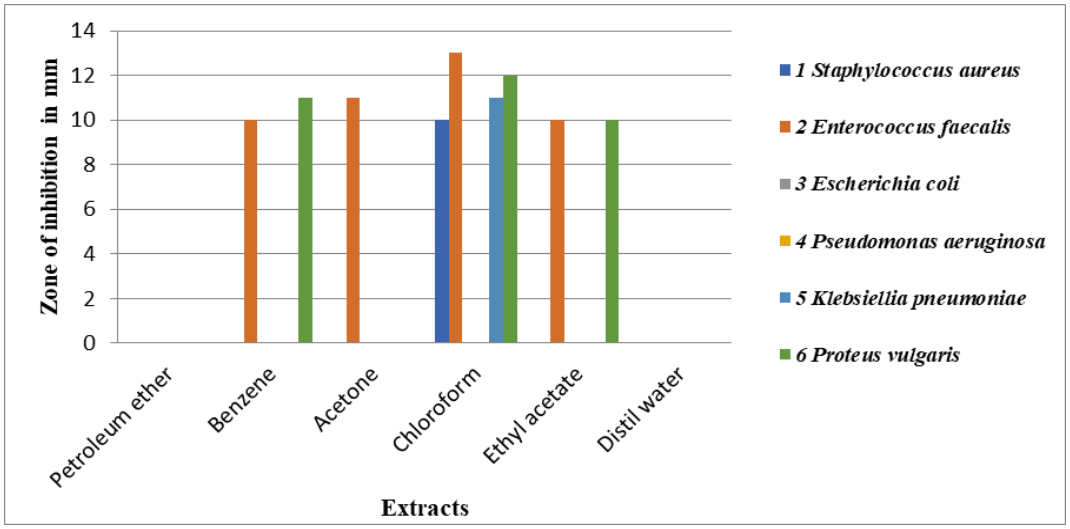

Figure 1: Analysis of antimicrobial sensitivity of root bark extract of *Strychnos potatorum* L. f.

Figure 2: Photography of *Strychnous potatorum* L.f

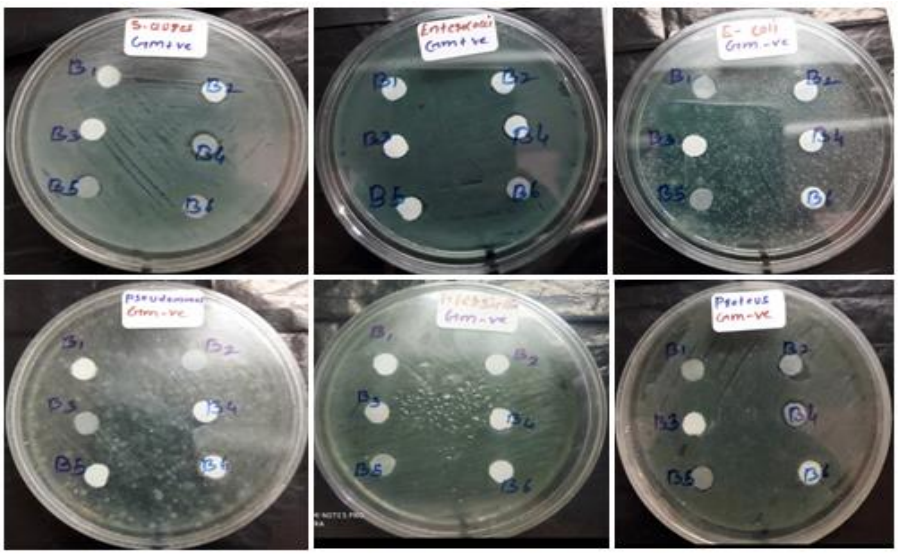

Figure 3: Antimicrobial sensitivity of root bark extracts of *Strychnos potatorum* L.f

The benzene extract showed inhibitory activity against *Enterococcus faecalis* with a zone of inhibition of 10 mm and *Proteus vulgaris* with a zone of inhibition of 11 mm. *Enterococcus faecalis* positive to the Acetone extract with a zone of inhibition of 11 mm. The Chloroform extract extract showed inhibitory activity against *Staphylococcus aureus* with a zone of inhibition of 10 mm, *Enterococcus faecalis* with a zone of inhibition of 13 mm, *Klebsiellia pneumoniae* with a zone of inhibition of 11 mm and *Proteus vulgaris* with a zone of inhibition of 12 mm. The extract in ethyl acetate showed positive result against *Enterococcus faecalis* and *Proteus vulgaris* with a maximum zone of inhibition of 10 mm. Terpenoids extracted in all solvent in moderate as well as abundant amount. Terpens have found to inhibit growth of cancerous cells and also decrease micro-organism concentration (Gupta *et al*.2011). The extract in petroleum ether, and distil water did not show any antimicrobial activity.

CONCLUSION

Strychnos potatorum, the multifaceted medicinal plant is the distinctive source of various types of compounds. Very little work has been done on the biological activity and probable medicinal applications of these compounds and hence investigation is needed to utilize their therapeutic utility to fight against diseases. Although crude extracts from various parts of *Strychnos potatorum* (nirmali) have medicinal applications from ancient time, modern drugs can be developed after extensive investigation of its bioactivity, mechanism of action, pharmacotherapeutics, toxicity, and after proper standardization and clinical trials. A deep research and development work should be undertaken on *Strychnos potatorum* (nirmali) and its products for their better economic and therapeutic utilization.

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