International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (1): 1332-1336
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved
UGC Approved Journal

Comparative Study of Antimicrobial Activity of *Soymida febrifuga* and *Saraca asoca* with Zitromax

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Received: 12 Oct 2018 / Accepted: 18 Nov 2018 / Published online: 1 Jan 2019
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Abstract

Natural plant products are the source of most active ingredients of the medicine. The extract of many plants uses in traditional medicine contain a wide range of curative agents that are used in many modern medicines. The present investigation is on phytochemical analysis and in vitro antimicrobial activities of dried leaves were done with the sample extracted with n-butanol and methanol extracts of *Soymida febrifuga* and *Saraca asoca*. From the phytochemical screening of the leaf extracts revealed the presence of carbohydrate, tannins, glycosides, flavonoids, phenols and steroidal compounds in the two plants. TLC profiles of both leaves extracts gives an idea about the presence of various phytochemicals. *In vitro* antimicrobial activity of both extracts was evaluated by disc diffusion method using some microbial species such as staphylococcus aureus, Escherichia coli, Bacillus substilis, klebsiella pneumonia, Pseudomonas aeuroginosa, candida albicans. The results of antimicrobial activity revealed that the extracts showed excellent inhibitory activity against all the tested pathogens and the both extracts showed comparatively better activity than zitromax.

Keywords
Antimicrobial activity, *Soymida febrifuga, Saraca asoca*, TLC, Phytochemicals, Plant extract.

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INTRODUCTION:

Nature has very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Traditional medicine has been improved in developing countries as an alternative solution to health problems and costs of pharmaceutical products. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to the host cells are considered for developing new antimicrobial drugs. Secondary metabolites such as flavonoids, alkaloids, glycosides, tannins and phenolic compounds have been established as the bioactive compounds of the plants. The aim of the present study is to screen in antimicrobial activity and phytochemical analysis of leaf extracts of medicinal plants of *Soymida febrifuga* and *Saraca asoca.*.

Soymida febrifuga (Roxb.) Juss is an indigenous lofty deciduous medicinal tree endemic to india, belonging to family meliaceae. Indian redwood is a huge tree bearing deciduous foliage and having a tough exfoliating in plates or scales. The compound leaves are crowded at the ends of branches. It contains some important constituents like in bark

lupeol, sitosterol, methyl angolensate, leaves contain quercetin, rutin and fruits abundantly contains tetraterpenoids. The decoction of bark has bitter resin principle well adapted for gargles, vaginal infections, enemata, rheumatic swellings, and stomach pain. It is also used for wounds, dental disease, uterine bleeding, haemorrhage and anticancer agent.

Ashoka is one of the most legendary and sacred trees of India. Asoca tree, universally known by its binomial latin name *Saraca asoca* belonging to Family Caesalpinaceae. It is handsome, small erect ever green tree with deep green leaves growing in dens clusters called in English Ashok tree. The ashoka is prized for its beautiful foliage and fragrant flowers. They are bright orange yellow in color, turning red before wilting. The ashoka tree is considered sacred throughout the Indian subcontinent, especially in India, Nepal and srilanka. It is also known as kankeli (Sanskrit), Ashoka (Assamese), Ashokadamara (Cannada), Ashokam (Malayalam), Ashokapatta (Telugu). It is especially sacred to the Hindu god of love, Kamadeva. The Indian philosopher and founder of Buddhism, Gauthama Siddhartha (c.563-483 B.C) was said to have been born under this tree. The aim of the present study is to provide complete information about the medicinal and pharmacological importance of the *soymida febrifuga* and *saraca asoca.*

***Soymida febrifuga*:**

- Kingdom : Plantae
- Division : Triacheophyta
- Class : Magnoliopsida
- Family : Meliaceae
- Genus : Soymida
- Species : Febrifuga

Common name: redwood, rohuna

***Saraca asoca*:**

- Kingdom : Plantae
- Family : Caesalpinaceae
- Division : Magnoliophyta
- Class : Magnoliopsida
- Genus : saraca
- Species : Asoca

MATERIAL AND METHODS:

The various grades of chemicals are used during experiments such as methanol, ethanol, DimethylSulfoxide(DMSO), con. Sulphuric acid, butanol, petroleum ether, Zitromax (standard), etc.

Methodology: -

Collection, of plant material.

The leaves of *Soymida febrifuga* and *Saraca asoca* collected locally from Gondia and authenticated with the help of authentic herbarium species. The leaves were shade dried for 7 days, coarsely powdered and stored in well-stoppered container. The dried material of both plants was then used further work.

The extraction of leaves of soymida febrifuga and saraca asoca:-

The coarsely dried powdered (300 g) leaves of *Soymida febrifuga* and *Saraca asoca* were extracted for solvents water reflux extraction successively in Soxhlet apparatus with butanol and methanol at a temperature 45 - 50° C for 5 – 6 hr. The evaporation of solvent up to 1/3 of original volume to get a concentrated extract. Liquid-liquid fraction distillation of concentrated extract with petroleum ether (100 ml:100 ml) for 4-5 time at a temperature 60° - 80°C to get highly concentrated extract. These extract transfer in disc and evaporated at 50°C give its form concentrated semisolid greenish gummy.The residue so obtained were subjected to preliminary phytochemical screening.

Phytochemical screening:

Test of sterol:

Salkwaski reaction: -Few mg of the residue of extract was taken in 2 ml of chloroform and 2 ml of con. Sulphuric acid was added from the side of the test tube. The test tube was shaken for few minutes. The development of red colour in the chloroform layer indicates the presence of sterols.

Test for alkaloids:

Dragendroff's reagent (Potassium bismuth iodide):- The above Dragendroff's reagent was sprayed on Whatman no. 41 filter paper and the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and the chloroform extract was applied on the filter paper, impregnated with Dragendroff's reagent, with help of an orange red paper on the paper indicates the presence of alkaloids.an orange red paper on the paper indicates the presence of alkaloids.

Test for glycoside:

Bornstrager's Test: - The extract was boiled with dilute sulphuric acid, filtered and to the filtrate, toluene or ether was added and shaken well. The organic layer was separated to which ammonia was added slowly. The ammonical layer shows pink to red colour due to presence of anthraquinone glycoside.

Keller-killiani test: - The test consist of boiling about 1 gm of extract with 10 ml 70% alcohol for 2-3 min. The extract was filtered. To the filtrate was added, 5 ml water and 0.5 ml strong solution of lead acetate

were, shaken well and filtered. The clear filtrate was treated with equal
volume of chloroform and evaporated to yield the extractive. The extractive was dissolved in glacial acetic acid and after cooling, 2 drop of ferric chloride solution was added to it. These contents were transferred to a test tube containing 2 ml con. Sulphuric acid.A reddish brown layer acquiring bluish-green colour after standing indicate the presence of cardiac glycosides.

Test for tannins:

Ferric chloride reagent: 5 % w/v solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. dark green colour indicate condensed tannin and deep blue colour indicate hydrolysable tannin.

Test for flavonoids (shinoda test):

A small quantity of test residue was dissolved in 5 ml ethanol (95 % v/v) and treated with few drops of con. hydrochloric acid and 0.5 gm of magnesium metal. The pink, crimson or magenta red colour are developed with in a minute or two if flavonoids are present.

Test for carbohydrates:

Fehling's test: -A little of the test residue was dissolved in water and a few ml of a Fehling's solution was added to it. This mixture was then warmed. If a red precipitate of cuprous oxide is obtained, reducing sugar are present.

Test for amino acid:

Ninhydrine test: - The ninhydrin reagent is 0.1 % w/v solution of ninhydrin in n-butanol. A little of this reagent was added to the test extract. A violet or purple colour is developed if amino acid is present.

Antimicrobial Activity: (by pour plate method)

Prepare nutrient agar Petri plates for the growth of bacterial cultures. Pour the cultures in agar media. The test cultures used such as Staphylococcus aureus, Bacillus subtilis and Escherichia coli. Prepare well in seeded plates by using cork borer that is sterile by burning with absolute ethanol. Plant extract 1 ml of (0.1 mg/ml) are added in the labeled well and incubated. One well is prepared as control using zitromax (Azithromycin) having 1 ml of (0.1 mg/ml) of pure solvent Dimethylsulfoxide (DMSO). Bacterial test culture plates are incubated at 32-37 ^{0}C for 48 hrs.

Minimum Inhibitory Concentration (turbidity method):

Prepare nutrient broth test tubes and label. In first tube (UT), inoculums is not added which is used for checking the sterility of medium and as a negative control. Other all test tubes, inoculums (three to four drops) is added to reach the final concentration of microorganism is 10^6 cell/ml. In all test tubes, test antimicrobial compound is added about o.1 to 1.0 ml except uninoculated (negative control) and control (positive) tube. The positive control tube is used to check the suitability of the medium for growth of the test microorganism and the viability of the inoculums. Adjust the final volume (10 ml) in all test tube by using sterile water. All test tubes are properly shaken and then incubated at 37^{0}c for two day.

Thin Layer chromatography:

The pharmacologically active butanolic and methanolic extract obtained from leaves of *S.febrifuga* and *S.asoca* was subject to thin layer chromatography to find out number of compound present in it.

RESULT AND DISCUSSION:

Table 1: Antibacterial result of *S. Febrifuga* and *S. Asoca:*

Sr. No.	Test Culture	Positive control (0.1mg/ml)	Zone of inhibition in (mm)			
			DMSO Solvent	Positive Control	*S. feb.* (s1)	*S.aso.* (s2)
1	B. subtilis	Zitromax	-	32	29	26
2	E. coli	Zitromax	-	23	21	19
3	S.aureus	Zitromax	-	34	23	27

The Methanolic and Butanolic extracts showed good antibacterial activity against Bacillus subtilis with 26 mm and 29 mm and moderate antibacterial activity against S.aureus with 23 mm and 27 mm and mild to E.coli with 21 mm and 19 mm.

Observation:

B. subtilis S.aureus E.coli

Fig.1. Observation of antibacterial activity by pour plate method:
C: - DMSO Solvent P: - Positive control (zitromax)
S1: -Sample (Butanolic extract) S2: -Sample (Methanolic extract)

Table 2: Minimum Inhibitory Concentration of *S.febrifuga* and *S. asoca*:

Sr. no.	Microorganism		Concentration(µg/ml)										MIC (µg/ml)
			50	100	150	200	250	300	350	400	450	500	
1	*S.feb.*	S.aureus	+	-	-	-	-	-	-	-	-	-	100
		E. coli	++	+	-	-	-	-	-	-	-	-	150
2	*S.aso.*	S.aureus	-	-	-	-	-	-	-	-	-	-	50
		E. coli	+	-	-	-	-	-	-	-	-	-	100

Turbidity: Present= (+); Absent = (-)
MIC value of *S. febrifuga* for S.aureus and E.coli was shows to 100 µg/ml and 150 µg/ml, while MIC of *S.asoca* was shows to 50 µg/ml and 100 µg/ml.

Table 3. Observation TLC of *S.febrifuga* and *S.asoca*:

Solvent system	UV Light	No.of component	*S.feb.* Rf value	*S.aso.* Rf value
Methanol: Water (8:1)	366 nm	1	0.79	0.61
Ethanol: Acetic acid (5:5)	366nm	1	0.93	0.47
Ethyl acetate: Chloroform (9:1)	366 nm	1	0.42	0.77

CONCLUSION:
S. febrifuga and *S. asoca* extracts was found to be an effective antimicrobial agent against bacterial pathogen. The evaluation of antimicrobial activities of both plants would plays a significant role for the findings of their more chemical entities and their bioactivities. From these results, the antimicrobial activity of S. *febrifuga* and *S. asoca* leaves extracts was shown effective and efficient result compared to standard against bacterial pathogens used.

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