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STUDY ON MORPHOLOGICAL, PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL ACTIVITIES OF *SARACA INDICA L. (THAW-KA)*

Htay Htay Myint[1], Nwe Nwe Aye[2]

Abstract

The medicinal plant *Saraca indica* L. belongs to the family Caesalpiniaceae. This plant is known as Thaw-ka in Myanmar. The specimens were collected from Dagon University, East Dagon Myothit Township, Yangon Region, during the flowering and fruiting period, February to June, 2019. In this research morphological characters, preliminary phytochemical tests and antimicrobial activities tests were studied. In the morphological study, the plant was small tree, dark green leaves and grey to dark brown barks. The leaves contain glycoside, tannin, steroids, carbohydrate, α-amino acid, alkaloid, saponin, phenolic compound and terpenoid. Reducing sugar, and starch were absent. The antimicrobial activities were carried out by agar well diffusion method on six types of test microorganisms. Ethanol extract and acetone extract showed the significant activities on all test microorganisms. Methanol extract showed the moderate against on all test organisms and ethyl acetate, chloroform and pet ether extracts showed the negative results for all types of test microorganisms.

Keywords : Morphological characters, Phytochemical test, Antimicrobial activities

Introduction

Advance in modern science and technology has contributed to an enormous development in the quality of human life. Though, stress in incidence of variety of psychiatric disorders. Drugs currently used in treatment of different neuropsychiatric and neurological disorders like anxiety, depression, schizophrenia, epilepsy, parkinsonism either refractory or have serious side effects or posses unfavorable drug-drug/ drug-food interactions. Plants are as medicine since time immemorial. Drugs from plant sources are being used by about 80 % of the world population. Herbal medicines have stood the test of time for their safely, efficacy, acceptability and lesser side effects.

The plant *Saraca indica* L. (Thaw-ka) belongs to the family Caesalpiniaceae. The common names of the plant are ashoka, asoka, asoka-tree, sorrowless tree of India (English), asok, ashoka, asupala, ashogam (Hindi); vànganh Lánh'o (Vietnamese). The native of the plant is India, Indonesia, Laos, Malaysia, Thiland and Myanmar. The word "Ashoka" means "no grief" in Sanskrit. The flowering season is around February to May. This plant is valued for its attractive foliage and fragrant flowers. It requires full sun or slight shade and soils from slightly acidic to neutral rich of organic substance, well drained and kept humid, adult plants can stand short dry periods. It can be cultivated in pot for the decoration of luminous greenhouses and winter gardens with lowest winter temperatures not under the 14°C.

The plant has been greatly used as traditional medicine for women related problems such as menorrhagia, leucorrhoea, bleeding hemorrhoids, dysfunctional uterine bleeding etc (Mohammad *et al.*, 2017). Parts of the tree used in traditional Ayruvedic medicine and homeopathic therapies. Juice obtained from boiling of bark said to be effective against female medicinal disorders like menstrual irregularities. Flowers are eaten against dysentery (website I).

Leaves are useful in stomach pain, help to remove worms from the stomach and thus provide relief from pain and swelling. Bark is used to prepare cosmetics that help to improve

[1] Dr, Lecturer, Department of Botany, Dagon University
[2] Dr, Lecturer, Department of Botany, Dagon University

skin complexion. The bark of the tree also has anti-fungal, anti-bacterial and pain relieving properties. The bark decoction help to treat internal piles. Flowers controls blood loss in stools. Seeds powder helps to control kidney stones. Fruits are used as a masticating kidney stones. Fruits are used as a masticating as a replacement for betel nuts. The wood is used in the buildings, the fruit as fodder and from the flowers get a dye.

The aim and objectives of the present research were to verify the morphological characters of this plant, to know the phytochemical constituents, medicinal uses and to examine the antimicrobial activities of leaves.

Materials and Methods

Botanical Studies

The specimens used in this research were collected from Dagon University, East Dagon Township, Yangon Region. They were collected especially during the flowering and fruiting period from January to June in 2018. The collected fresh specimens of both vegetative and reproductive parts of the plants were identified by using literatures of Lawrences, 1964; Backer, 1965; Hundley and Chit Ko Ko, 1987; Dassanayake, 2000 and Kress *et al.*, 2003.

Chemical Studies

The leaves of *Saraca indica* L. were collected from Dagon University, East Dagon Township, Yangon Region. The leaves samples were washed with water and were cut slices by knife. Then these slices were dried at room temperature for 2-3 weeks. The dried leaves were pulverized by grinding with a blender to get fine powdered and stored in air tight container. For preliminary phytochemical test, the air-dried powdered of the leaves were tested for alkaloids, α-amino acid, glycoside, cyanogenic glycoside, carbohydrates, reducing sugar, starch, saponin, tannin, phenolic compound, steroids and terpenoids were carried out.

Preliminary Phytochemical Test of Leaves of *Saraca indica* Linn.

The preliminary phytochemical tests were carried out according to Vogel, 1956; British Pharmacopoeia 1968, Marini Bettolo *el. al.,* 1981; Robinson 1983 and Central Council for Research in Unani Medicine, 1987.

Test for Alkaloid

One gram of powdered sample was boiled for about 20 minutes with 20ml of 10% HCl and filtered. The filtrate was divided into three portions and tested with Dragendroff's reagent, three Wagener's reagent and Mayer's reagent. The precipitate formed an addition of the reagent indicated the presence of alkaloid (Robinson, 1983).

Test for α-Amino acid

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and then filtered. And then, a few drops of each filtrate was spotted on a filter paper using a capillary tube, allowed it to dry and sprayed with ninhydrin reagent. The filter paper was dried at room temperature and then kept it in over at 110°C for a few minutes after which the purple colour appears due to the presence of α-amino acids (Marini Bettolo *et. al.,* 1981).

Test for Glycoside

One gram of powdered sample was heated in a glass test tube with 10ml of distilled water on the water-bath for 20 minutes. The mixture was filtered and 10% basic lead acetate solution was added drop-wise to the filtrate. Pale yellow precipitate was observed which showed the presence of glycoside (Marini Bettolo *et. al.*,1981).

Test for Carbohydrate

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and filtered. The filtrate was introduced into a test tube and a few drops of 10% α-naphthol was added shaken. The test tube was then inclined at an angle of 45° and concentrated sulphuric acid was added slowly along the side of the tube. A red ring was formed between the two layers, showing the presence of carbohydrate (Marini Bettolo *et al.,* 1981).

Test for Reducing Sugar

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and filtered. The filtrate was treated with Fehling's solution, then boiled about 20 minutes, it furnished green precipitates, indication the presence of a reducing sugar (Vogel, 1956).

Test for Starch

One gram of dried powdered sample was boiled with 10 ml of distilled water for about 20 minutes. It was then filtered and two drops of iodine solution were added to the filtrate. Blue black colour was formed which indicate the presence of starch (Marini Bettolo *et. al.,* 1981).

Test for Saponin

One gram of powdered sample was boiled with 10 ml of distilled water for about 20 minutes and filtered. The filtered and the filtrate shaken vigorously with distilled water for a few minutes. Market forthing which lasted for about half an hour to take place, indicating the presence of saponin (Marini Bettolo *et al.,* 1981).

Test for Tannin

One gram of powdered sample was boiled with 10ml of distilled water for about 20 minutes and filtered. The filtrate was treated with a few drops of 1% ferric chloride solution. If a bluish black or yellowish brown colour resulted indicating the presence of tannins (Marini Bettolo *et al.,* 1981).

Test for Phenolic compound

One gram of powdered sample was boiled with 10 ml of distilled water for 20 minutes and filtered. The filtrate was treated with neutral *5%* ferric chloride solution, it gave deep blue colour, indicating the presence of phenol groups (Marini Bettolo *et. al.,* 1981).

Test for Steroids and Terpenoids

One gram of powdered sample was extracted with petroleum ether for 20 minutes and filtered. When the petroleum ether extract was dissolved in 1ml chloroform. The chloroform extract was treated with 3 drops acetic anhydride and concentrated sulphuric acid. The reenish colour was turns to blue green indicate the presence of steroids and deep pink of terpenoid (Central Council for Research in Unani Medicine, 1987).

Antimicrobial Activities of Different Solvent Extracts from Leaves of *Saraca indica* L.

Apparatus Used

Autoclave, clean bench, conical flask, hot air sterilizer, measuring cylinders, micropipettes, steam-drying oven, petridishes, pipettes, water bath and loops.

Extraction of crude drugs

Five grams of powder was soaked with 50ml of different solvent such as ethyl acetate, chloroform, methanol, ethanol, acetone, water and pet-ether for about three days and thoroughly shaked. The mixture was filtered and evaporated.

Aseptic Techniques

Sterilized Pyrex glass- wares used throughout the experiment. Glass- wares were first acid washed and then rinsed in distilled water and were sterilized by using autoclave at 121°C for 15 minutes. Once substances are sterilized, they stay sterile as long as they remain within containers that do not permit living organisms to enter. But in research works, the culture materials are transferred from one container to another. The methods that do not permit the accidental entry of living microorganisms during the transfer process were collectively called as aseptic technique. Inoculation wires, the mouth of the test tubes, flasks and culture bottles are heated during transferring process, and working quickly in a clean area were as aseptic techniques.

Cultivation of Test Organisms

Bacillus subtilis (N.C.T.C-8236), *Staphylococcus aureus* (N.C.P.C-6371), *Pseudomonas aeruginosa* (6749), *Bacillus pumilus*(N.C.I.B-8982), *Candida albicans and Escherichia coli* (N.C.I.B-8134*)* were used for the antimicrobial activities. They were inoculated into the nutrient broth and transferred into nutrient agar media.

Preparation of Culture Media

Nutrient Agar (NA) Medium (Atlas, 1993)

Nutrient Agar	-	28.0 g
Agar	-	16.0 g
Distilled Water	-	1000 ml
pH	-	6.8

After autoclaving, Nystatin 1.5 ml was added to the medium for bacteria and Chloramphenicol was added to the medium for fungi.

Nutrient Broth Medium (Atlas, 1993)

Peptic digest of animal tissue	-	5.0 g
Beef extract	-	1.5 g
Yeast extract	-	1.5 g
Sodium chloride	-	5.0 g
Distilled water	-	1000 ml
pH	-	6.8

After autoclaving, Nystatin 1.5ml was added to the medium for bacteria and Chloramphenicol was added to the medium for fungi.

Preparation of Plates for Antimicrobial Activities Test

The antimicrobial activities were performed by agar-well diffusion method. Nutrient agar was prepared according to method described by Cruikshank, 1975. Nutrient agar was boiled and 20-25 ml of the medium was poured into a test- tube and plugged with cotton wool and autoclaves at 121 °C for 15 minutes. Then the tubes were cooled down to 30-35°C and poured into sterilized petridishes and 0.01 ml of spore suspension were also added into the dishes. The agar was allowed to set for 30 minutes after with 5mm plate agar well was made with the help of sterilized cork borer. After that, about 0.1ml of sample was introduced into the agar-well and incubated at 37°C for 24-48 hrs. The inhibition zone appeared around the agar-well indicating the presence of anti-microbial activity. The extent of antimicrobial activity was measured from the zone of inhibition diameter. The results were shown in Table 2, Figures 4 to 5.

Results

Morphological Chracters of *Saraca indica* L.

Scientific name	-	*Saraca indica* L.
Myanmar name	-	Thaw-ka
English name	-	ashoka, asoka, sorrowless tree
Family	-	Caesalpiniaceae
Flowering and fruiting period	-	February to June
Parts used	-	Leaves

Small trees, evergreen about 6 m in high. Stems are cylindrical, woody and greywish-brown. The leaves are alternate, paripinnately compound, petiolate, stipulate; Leaflets are elliptic-lanceolate, 6.5-24-5 cm, rounded at the base, margin entire, acuminate apex, both surfaces shinning and dark green; petioles are cylindrical, 2.0-3.0 cm; 0.4-0.5 × 0.2-0.3 cm; stipules are lanceolate about 0.6 × 0.3 cm, caducous. The inflorescences are axillary, corymb racemose; peduncles are cylindrical, about 1.5-2.5 cm long, reddish green, glabrous, The flowers are yellow to about 2.5 cm in diameter at anthesis, bracteate, bacteolate, pedicellate, complete, bisexual, regular, actinomorphic, tetramerous, hypogynous; bract ovate, about 0.6 × 0.6 cm, pale red; bracteolate ovate, about 0.4 × 0.4 cm, pale red; pedicel are cylindrical, about 0.6 × 0.2 cm and red. The sepals are 4, synsepalous, calyx tube about 2.2 × 0.4 cm, calyx lobe about 1.2 × 1.2 cm, valvate, petaloid (yellow to red) persistent, pubescent, inferior. The petals are absent. The stamens are (6-7), fuse, episepalous, filament about 3.0 cm, filiform, staminal tube very short; the anther is dithecous, about 0.2-0.4 cm, purple, extrorse, dorsifixed, longitudinal dehiscence, inferior. The ovary is superior, oblong, compressed, about 0.2 cm in diameter, slightly curved, borne on the stalk, adnate to the calyx tube, pink, carpel 1, monocarpellary, apocarpous, uniloeular, marginal placentation, one ovule in each locule in T.S; hairy; style is one, terminal, about 2.8 cm long, red, glabrous; stigma is capitate, gynophore present, about 2.5 cm. The fruits are legume, oblong, 1.2 × 4.0 cm and green. The seeds are 5, ovoid and about 3.0 × 2.2 cm.

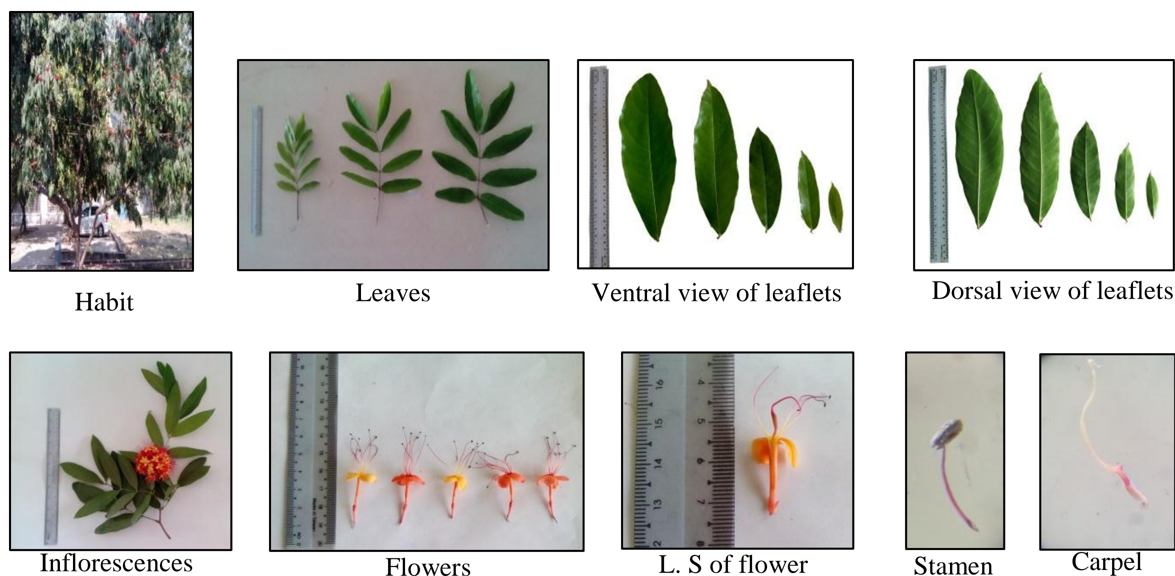


Figure 1 Morphological characters of ***Saraca indica* L.**

Preliminary Phytochemical Test of Leaves from *Saraca indica* L.

The results of preliminary phytochemical test of air-dried powdered leaves from *Saraca indica* L. indicated that tannin, steroids, carbohydrate, α-amino acid, alkaloid, saponin, phenolic compound and terpenoid were found to be present and reducing sugar and starch were absent. Among them, the amount of precipitate from glycoside was highest than the other tests.

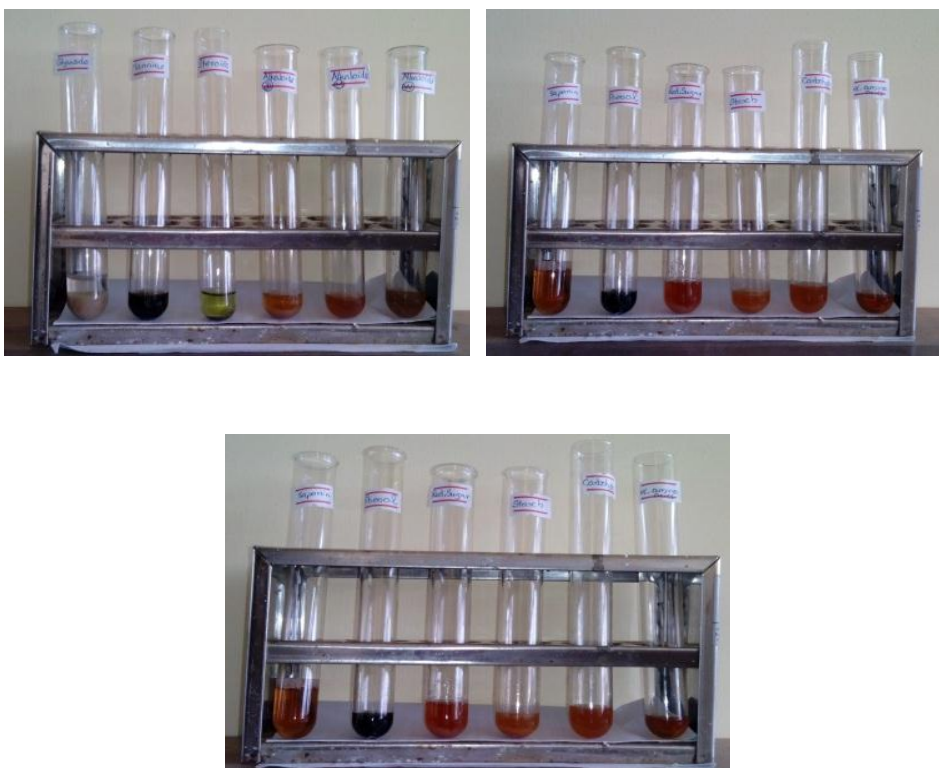


Figure 2 Phytocehmical Test of Leaves from *Saraca indica* L.

Table 1 Preliminary Phytochcmical Test of Leaves from *Saraca indica* L.

No.	Test	Extract	Test reagent	Observation	Results
1.	Alkaloid	10% HCl	Dragendroffs reagent Wagner's reagent Mayer's reagent	Orange ppt Brown ppt White ppt	+ + +
2.	α-amino acid	H_2O	Ninhydrin reagent	Pink spot	+
3.	Glycoside	H_2O	10% lead acetate solution	White ppt	+
4.	Carbohydrates	H_2O	10% α-napthol + H_2S0_4 (Conc:)	Red ring	+
5.	Reducing sugar	H_2O	Fehling's solution	ppt	+
6.	Starch	H_2O	Iodine solution	Brown ppt	+
7.	Saponin	H_2O	Distilled water	Foaming	+
8.	Tannin	H_2O	1% $FeCl_3$ solution	Bluish black	+
9.	Phenolic compound	H_2O	5% $FeCl_3$ solution	Deep blue	+
11.	Steroids	Petroleum ether	Acetic anhydride	Green ppt	+
12.	Terpenoids	Petroleum ether	Acetic anhydride and H_2S0_4 (Conc:)	Black ppt	+

Key to the table

Key to the table (+) = present (-) = absent (ppt.) = precipitate

Antimicrobial Activities of Different Solvent Extracts of Leaves of *Saraca indica* L.

In this study, ethanol and acetone extract showed the significant activities on six test organisms. Methanol extract showed the moderate against on all test organisms. Ethyl acetate, chloroform end petether extract the negative results for all types of test organisms.

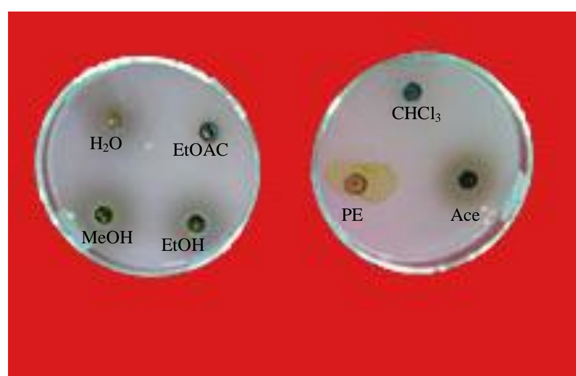
Figure 3 Leaves extracts of ***Saraca indica*** **L.**

Table 2 Inhibition Zone Exhibited by Different Solvent Extract of Leaves of ***Saraca indica*** **L.**

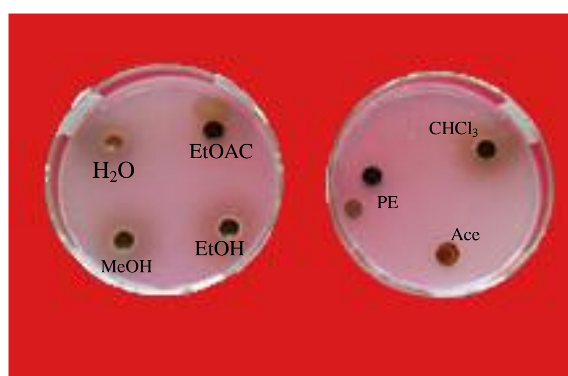
No.	**Microorganisms**	**Inhibitions Zone (mm)**						
		H_2O	MeOH	EtOH	EtOAC	$CHC1_3$	P.E	Ace
1.	*Pseudomonas aeruginosa*	-	25 ++	25 ++	-	-	-	25 ++
2.	*Staphylococcus aureus*	25 ++	20 +	25 ++	-	-	-	25 ++
3.	*Candida albicans*	30 ++	25 ++	20 +	-	-	-	25 ++
4.	*Esherichia coli*	-	20 +	30 ++	-	-	-	25 ++
5	*Bacillus subtilis*	15 +	25 ++	25 ++	-	-	-	25 ++
6.	*Bacillus pumilus*	25 ++	25 ++	25 ++	-	-	-	25 ++

Key to the table

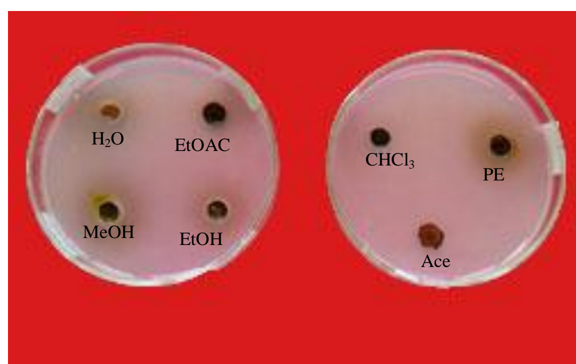
(+) = 10mm-20mm (++) = 21mm-30mm (+++) = 31mm above (10 mm) = Agar well



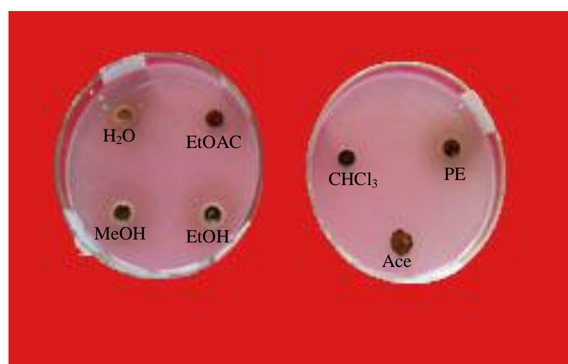
Pseudomonas aeruginosa



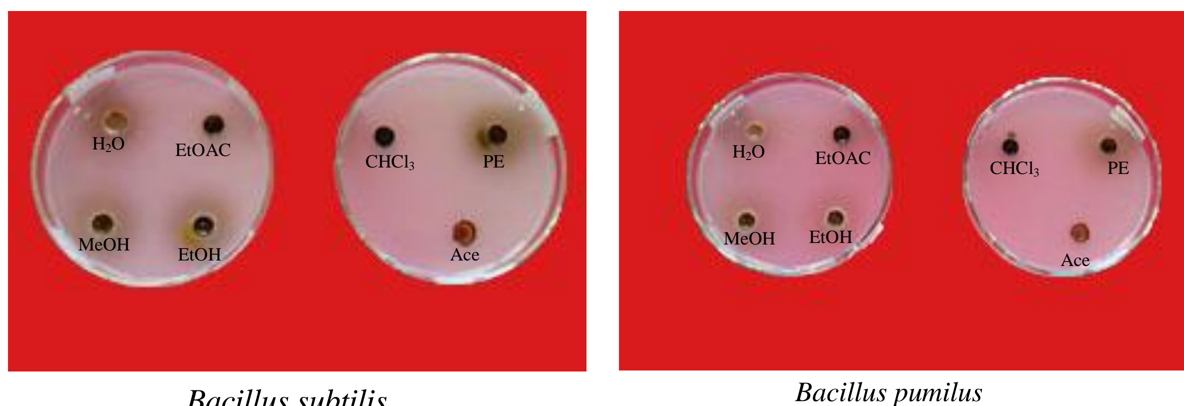
Staphylococcus aureus



Candida albicans



Esherichia coli



Bacillus subtilis *Bacillus pumilus*

H_2O = Aqueous

MeOH = Methanol

EtOH = Etanol

EtOAc = Ethyl acetate

$CHCl_3$ = Chloroform

P.E = Petroleum ether

Ace = Acetone

Figure 5 Antimicrobial activities of leaves of *Saraca indica* L.

Discussion and Conclusion

Herbal medicine has such as amazing influence that various alternative medicine therapies with herbal remedy, Unani and Ayurveda. The tree has many heath benefits and has long be used in traditional Indian medicine.The medicinal importance of the tree is one of the most important medicinal plant which possess a lot of therapeutic values especially for female disorders.

The plant *Saraca indica* L. belongs to the family Caesalpiniaceae. The plants are small trees. The leaves are alternate, paripinnately compound, petiolate and stipulate. Leaflets are elliptic-lanceolate, rounded at the base, margin entire, acuminate apex, both surfaces shinning, dark green. The stipules are lanceolate and caducous. The inflorescences are axillary, corymb racemose, peduncles are cylindrical, reddish green and glabrous. The flowers are yellow to red, bracteate, bracteolate, pedicellate, complete, bisexual, regular, actinomorphic, tetramerous and hypogynous. The sepals are yellowish to red. The petals are lacking. The stamens are fused. The filaments are filiform and distinct. The anthers are dithecous. The ovary is superior and oblong. These datas were agreed with Dassanayake, 1991.

The primary benefit of *Saraca indica* L. is a brain tonic and improve memory and intellect. It can be used to treat epilepsy and headache. It also controls vomiting and help to cure diabetes. The herb of this tree can acts on uterine muscles and endometrium and thus provides relief from abdominal pain and other spasms. It also helps to treat irregular menstrual cycles, amenorrhea, leucorrhea, fibroids, cysts and other related disorders. This tree is widely used to treat gynecological and menstrual problems in women and it helps to remove toxins from our blood and therefore provides excellent benefits for our skin. (website 1).

The flowers were eaten by cooked, aromatic, with a somewhat sour flavour. Eaten as potherb. Fruits are used as masticatons as a replaced for betel nuts. Although the health benefits of this tree are numerous, pregnant women should abstain from consuming products from this tree as it might lead to complications.

In this research, the preliminary phytochemical screening of the extracts of leaves contain glycoside, tannin, steroids, carbohydrate, α-amino acid, alkaloid, saponin, phenolic compound and terpenoid. These results were agreed with Aditya *et al*., 2013 and Mohammad *et al.,* 2017.

In the antimicrobial activities test, six types of test organisms were used. Among them, ethanol and acetone extract of leaves showed the best significant activities on all test organisms. Methanol extract showed the moderate against on all test organisms and ethyl acetate, chloroform and pet-ether extract showed the negative results for all types of test microorganism. These datas were agreed with Aditya, 2013.

Therefore, the present study focused on chemical composition by using preliminary phytochemical test and antimicrobial activities of this plant which could be assumed to be beneficial for human health.

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