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RESEARCH ARTICLE

COMPARATIVE STUDIES ON THE ANTIMICROBIAL ACTIVITY OF WATERY LEAFY EXTRACTS FROM *LAWSONIA INERMIS* L.(HENNA), *OROXYLUM INDIUM* (L.)VENT (MIDNIGHT HORROR) AND *MELASTOMA MELABATHRICUM* L. (MALABAR)

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ABSTRACT

In the present investigation the antimicrobial activities of some traditional medicinal plants which were selected based on medicinal reports practiced by native people of Myeik Township, Tanintharyi region in Myanmar. Antimicrobial activities of aqueous extract of *Lawsonia inermis* L.(Henna), *Oroxylum indium* (L.)Vent (midnight horror) and *Melastoma melabathricum* L.(Malabar) tested against the following microorganism like *Staphylococcus aureus, Bacillus pumilus* ,*Bacillus subtilis* , *Pseudomonas aeruginosa* ,*Escherichia coli* and *Candida albicans* by Agar-well diffusion method. The *Lawsonia inermis* L. showed maximum zone of inhibition against *Staphylococcus aureus*, (15 mm) and minimum (13 mm) of *Candida albicans*. The *Oroxylum indium* (L.)Vent showed maximum zone of inhibition against *Staphylococcus aureus*, (13 mm) and minimum (11 mm)of *Pseudomonas aeruginosa*. *Melastoma melabathricum* L. showed maximum zone of inhibition against *Bacillus subtilis* (13 mm) and the minimum (12 mm) of *Candida albicans*. The results revealed the presence of important medicinal phytochemicals constituents, such as alkaloids, flavonoids, phenols, saponins, α-amino acids, reducing sugar, phlobatannins and tannins. were present in the *Lawsonia inermis* L. and *Melastoma melabathricum* L.For *Oroxylum indium* (L.)Vent, presence of alkaloids, glycosides, phlobatannins, phenolic compounds, α-amino acids, saponins and tannins which interpret its medicinal values. Among the three samples, coumarins was found to be presence in *Lawsonia inermis* L.

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INTRODUCTION

Medicinal plants have been a major source of treatment for human diseases since time immemorial. One fourth of the world population i.e. 1.42 billion people are dependent on traditional medicines, particularly plant drug for curing ailments Herbal medicines are promising choice over modern synthetic drugs. They show minimum side effects and are considered to be safe (9). In this research work, the aqueous extract of *Lawsonia inermis* L.(Henna) leaves, *Oroxylum indium* (L.)Vent (midnight horror)leaves and *Melastoma melabathricum* L. (Malabar) leaves from Tanintharyi region in Myanmar which were used to traditional medicines of wound healing and hypertension treatment , were checked its authenticity and purity as per the standard parameter. *Lawsonia inermis*, also known as henna, the henna tree, the mignonette tree, and the Egyptian privet, (Bailey, 1976) is a flowering plant and the sole species of the *Lawsonia* genus. It is the source of the dye Dan used to dye skin, hair and fingernails, as well as fabrics including silk, wool and Leather (Okwu, 2006). The henna plant is native to northern Africa, western and southern Asia, and northern Australasia, in semi-arid zones and tropical areas. It produces the most dye when grown in temperatures between 35°C and 45 °C (95 and 113 °F) (Leal, 2000). Main chemical constituents of the Henna are Lawsone (2-hydroxynaphthoquinone), mucilage, mannite, gallic acid and tannic acid (Rubiay, 2008). Henna is known to be used as cosmetic agent for dyeing hair, nails and skin(20).In traditional medicine, Henna plant is used to treat many diseases like oedema, bronchitis, menstrual disorder, rheumatism, hemorrhoids and even injaundice, leprosy, pain, spleenen largement, dysentery and skin problems (Cuong, 2009). Henna can also be used as anastringent and antihemorragic agent and is also known for its hypotensive, cardio inhibitory and sedative effects (Warrier, 1995) .In addition, henna is reported to show some other properties including hypoglycemic immstimulant, hepatoprotective, antiinflammatory, tuberculostatic ,anticancer and antioxidant properties (Syamsudin, 2008).

Oroxylum indicum. (L.) (midnight horror) decoction is given in treating rheumatic pain, enlarged spleen (Khare, 2004), ulcer, cough, and bronchitis. Mature Fruits are acrid, sweet, anthelmintic, and stomachic. They are useful in pharyngodynia, cardiac disorders, gastropathy, bronchitis, haemarrhoids, cough, piles, jaundice, dyspepsia, smallpox, leucoderma and cholera (Kala, 2011). Seeds are used as purgative. Dried seed powder is used by women to induce conception. Seeds yield non-drying oil used in perfume industry. The seeds are ground with fire soot and the paste is applied to the neck for quick relief of tonsil pain. The seeds are used in traditional Indian Ayurvedic medicine, included in famous tonic formulations such as Chyawanprash (Olaleye, 2007). Bark decoction is taken for curing gastric ulcer and a paste made of the bark powder is applied for mouth cancer, scabies and other skin diseases. The medicated oil of O. indicum in sesame oil base instilled into ears mitigates the pain in otitis (Warrier, 1995) and fiber. Roots are sweet, astringent, bitter, acrid, refrigerant (Yoganarasimhan, 1996) antiinflammatory, anodyne, aphrodisiac, expectorant, appetizer, carminative, digestive, anthelmintic, constipating, diaphoretic, diuretic, antiarthritic, antidiabetic and febrifuges. Tonic is useful in dropsy, cough, sprains neuralgia, hiccough, asthma, bronchitis, anorexia, dyspepsia, flatulence, colic, diarrhea, dysentery, strangury, gout, vomiting, leucoderma, wounds, rheumatoid arthritis and fever. Root bark is used in stomatitis, nasopharyngeal cancer and tuberculosis (Bhattacharje, 2005). Leaves are used as stomachic, carminative and flatulent. Several workers have reported different biochemical activities of O. indicum in various in vivo and in vitro test models. Different part of this plant have been found to exhibit anti-inflammatory, antimicrobial, antioxidant, anticancer, anti-mutagenic, photocytotoxic, anti-arthritic, immunostimulant, hepatoprotective, anti-proliferative and hepatoprotective activities.

Melastomataceae (Malabar) plants originate in the tropic and subtropic regions, with a total of more than 4000 species in the world. In the Southeast Asian region alone, the genus Melastoma comprises 22 species, 2 subspecies, and 3 varieties (Rajenderan, 2010). In general, M. malabathricum is a small shrub commonly found in previously cleared land, waste places, and roadside throughout the Southeast Asian countries, including Tanintharyi region in Myanmar. It is native to tropical and temperate Asia and the Pacific Islands (Ling, 2009). The plant is one of the most common weeds that grow wildly and abundantly throughout the tropics, especially in the moist areas, and can be found in the Indian Ocean Islands, throughout South and South-East Asia, China, Taiwan, Australia, and the South Pacific Ocean (Wong, 2008). The leaves are chewed up, pounded, and applied as paste on cuts or wounds or finely chopped up and squeezed to apply the juice onto the wound to stop bleeding (Latiff, 2000). According to Sharma *et al*. ,the leaves can also be used to prevent scarring from smallpox, to treat dysentery, diarrhea, and piles, and as a tonic (Zakaria *et al*., 2006). The young leaves are eaten to treat diarrhea while the young premature leaves are consumed raw to cure dysentery (Koay, 2008). The shoots can be ingested to treat puerperal infections, high blood pressure, and diabetes while the shoots juice can also be used as a mouthwash to relieve a toothache or to treat leukorrhea. Other than those mentioned above, the leaves are also medicinally useful to treat ulcers, gastric ulcers, scar, pimple, and black spot at skin .The roots can also be used as mouthwash to relieve a toothache and to treat epilepsy (Burkill, 1996) given to postpartum women to aid healing and womb strengthening (Fazlin, 2002) or to alleviate rheumatism, arthritis, and tenderness in the legs (Koay, 2008) Other than that, the powdered leaves and roots can be applied to wounds and pox scars to aid the healing process (Fazlin, 2002) or used to relieve the discomfort of hemorrhoids with the former also used as astringent for dysentery (Sajem, 2006), (Strasser, 2010).

MATERIALS AND METHODS

Sample Collection: Fresh Leaves of *Lawsonia inermis* L.(Henna), *Oroxylum indium* (L.)Vent(midnight horror) and *Melastoma melabathricum* L.(Malabar)were collected from Myeik Township, Thanintharyi Division, Myanmar. The collected sample was identified in Department of Botany, University of Myeik.

Chemicals: All chemicals used in this work were from British Drug House Chemical Ltd., Poole, England. All standard solutions and other diluted solutions throughout the experimental runs were prepared by using distilled water. In all the investigations the recommended methods and standard procedures involving both conventional and modern techniques were employed (Vogel, 1978). All other chemicals and reagents used were of analytical grae.

Preparation of leaf extracts: The sample was cleaned from dust, washed with water, chopped, and dried at room temperature for one week. The dried material was made powder by using grinding machine, and stored in airtight glass bottle until used. The dry powdered sample material (15)g was extracted with 100ml of water ,ethanol, and methanol separately .The contents were kept as such in room temperature for 48h with constant stirring at regular intervals After the incubation period, the contents were filtered through Whatman No.1 filter paper. Then filtrate were vacuum dried using rotary evaporator and concentrates were stored at 4°C.The residues were redissolved with the appropriate solvents from which they were prepared and used for further studies.

Preliminary Phytochemical analysis: Qualitative phytochemical analyses were performed in filtrates of *Lawsonia inermis* L. (Henna), *Oroxylum indium* (L.)Vent (midnight horror) and *Melastoma melabathricum* L. (Malabar) preliminary phytochemical test were carried out according to determine the prescence of phytochemicals the alkaloid, α--amino acids, carbohydrates ,flavonoids, phenolic compounds, reducing sugar, tannin ,starch ,glycosides, saponins , coumarins and phlobatannins as described by standard procedure.

Elemental analysis: Elemental analysis of *Lawsonia inermis* L.(Henna) , *Oroxylum indium* (L.)Vent (midnight horror) and *Melastoma melabathricum* L. (Malabar) samples were determined by EDXRF method.

Test organism: Screening of Antimicrobial activity of *Lawsonia inermis L. (*Henna*), Oroxylum indium (L.)Vent (*midnight horror*) and Melastoma melabathricum* L. (Malabar) samples were determined by agar well diffusion method in DCPT, Yangon. Six species of microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albican* and *Escherichia coli* were treated with the sample.

Preparation of inoculums: The microorganisms were inoculated into nutrient broth and rose Bengal broth for bioassay and incubated For 24 and 48 h at 37°C.The turbidity of the medium indicates the growth of organisms.

Antimicrobial studies: The agar well diffusion method was employed for the determination of antimicrobial activity of extracts. Lawn culture of *E.coli, Candida albican, Bacillus puimilus, psrudomonus aeruginosa, Staphylococcus aureus and Bacillus subtils* were spread on nutrient agar and A. niger & A flavus spread on rose bengal agar using sterile cotton swabs. The wells (6mm in diameter) were cut from the agar plates using a cork horer.30µlof the extracts (7mg/ml)were poured into the well using a sterile micro pipette .The plates were incubated at 37±2°C for 24 hours for bacterial activity and 48 hours for fungal activity .The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

RESULTS AND DISCUSSION

In this research work, the aqueous extract of *Lawsonia inermis* L.(Henna) , *Oroxylum indium* (L.)Vent (midnight horror)and *Melastoma melabathricum* L.(Malabar) sample leaves from Tanintharyi region in Myanmar which were used to traditional medicines of wound healing and hypertension treatment, were checked its authenticity and purity as per the standard parameter.

Figure 1. The plant and leaves of *Oroxylum indicum*. (L.)Vent(midnight horror)

Figure 2. The plant and leaves of *Lawsonia inermis* L. (Henna)

Preliminary phytochemical profiling reflects essential information regarding the diversity of different classes of secondary metabolites such as alkaloids, flavonoids, steroids, saponins, tannins, reducing sugars etc. in the plant extracts.

Figure 3. The plant and leaves of *Melastoma melabathricum* L. (Malabar)

In the present study, qualitative tests for all three crude extracts showed significant indication about the presence of various secondary metabolites. Preliminary phytochemical screening of *Lawsonia inermis* L, *Oroxylum indium* (L.)Vent and *Melastoma melabathricum* L.leaves extracts revealed the presence of various bioactive compounds and the results were presented in Table 1 . The phytochemical results of *Lawsonia inermis* L.leaves extracts indicated positive for all except starch while *Oroxylum indium* (L.)Vent extracts tested positive for all except flavonoids, and coumarins. The phytochemical results of *Melastoma melabathricum* L. leaves extracts indicated positive for all except starch and coumarins. The people of Myeik in Tanintharyi Region also used the fresh and dry leaves of *Lawsonia inermis* L. and *Melastoma melabathricum* L. to treat anti-inflammatory and cuts and wounds.

This is due to the presence of saponin, flavonoids tannins and phlobatannins in *Lawsonia inermis* L leaves and *Melastoma melabathricum* L.leaves. The presence of saponins, and tannins were also reason why the leaves are used traditionally to use wound healing (Malinow, 1977). The juice from the *Lawsonia inermis* L. leaves and *Melastoma melabathricum* L. leaves have been found to be anti-inflammatory .This can be explained due to the presence of saponins and flavonoids as both constituent show anti-inflammatory properties (Kenner, 1996). The presence of phlobatannins in *Lawsonia inermis* L leaves and *Melastoma melabathricum* L. leaves which have been reported for its wound healing properties, these are anti-inflammatory and analgesic (Ayinde, 2007) and antioxidant (Okwu, 2004). Moreover, the presence of alkaloids and saponins in the leaves of *Oroxylum indium* (L.)Vent as shown in Table explains why the leaves of *Oroxylum indium* (L.)Vent used for hypertension treatment, mainly in Myeik Township, Tanintharyi Region (Akinpelu, 2006). Among the three samples, coumarins was found to be in Lawsonia inermis L. Many pharmacological activities have been ascribed to coumarins such as anticlotting, hypotensive, antimicrobial, anti-inflammatory, and antitumor activities .The results revealed the presence of important medicinal phytochemicals constituents, such as terpenoids, reducing sugar, flavonoids, alkaloids and phlobatannins were present in the *Lawsonia inermis* L. and *Melastoma melabathricum* L..For *Oroxylum indium* (L.) Vent, presence of alkaloids, glycosides, phlobatannins, phenolic compounds, α-amino acids, saponins and tannins which interpret its medicinal values. EDXRF spectrometer was used to verify the relative quantitative percentage of elements in the leaves of *Lawsonia inermis* L., *Oroxylum indium* (L.)Vent and *Melastoma melabathricum* L.

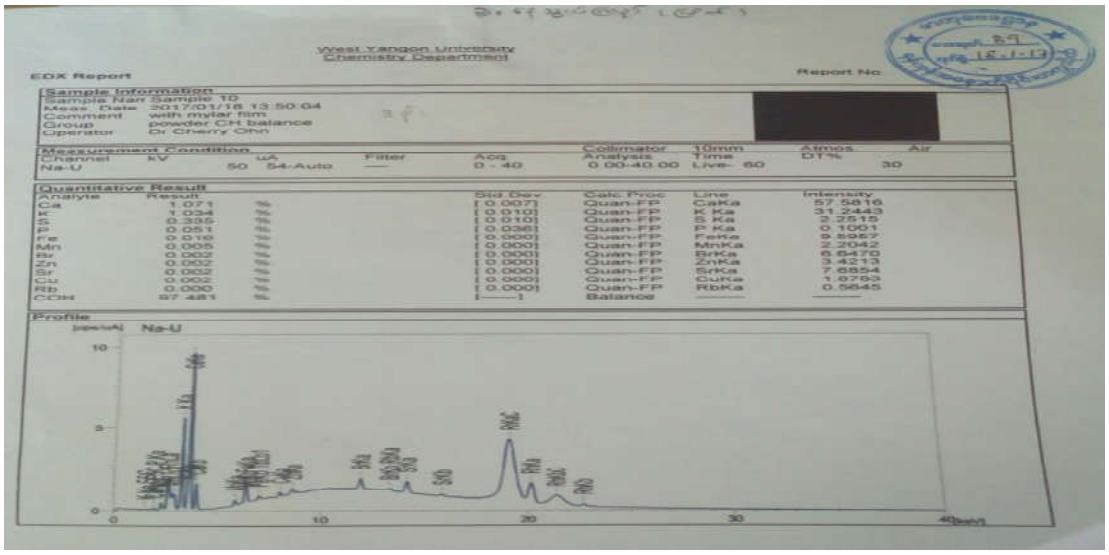

Figure 4. EDXRF spectrums of *Lawsonia inermis* L. (henna) leaf

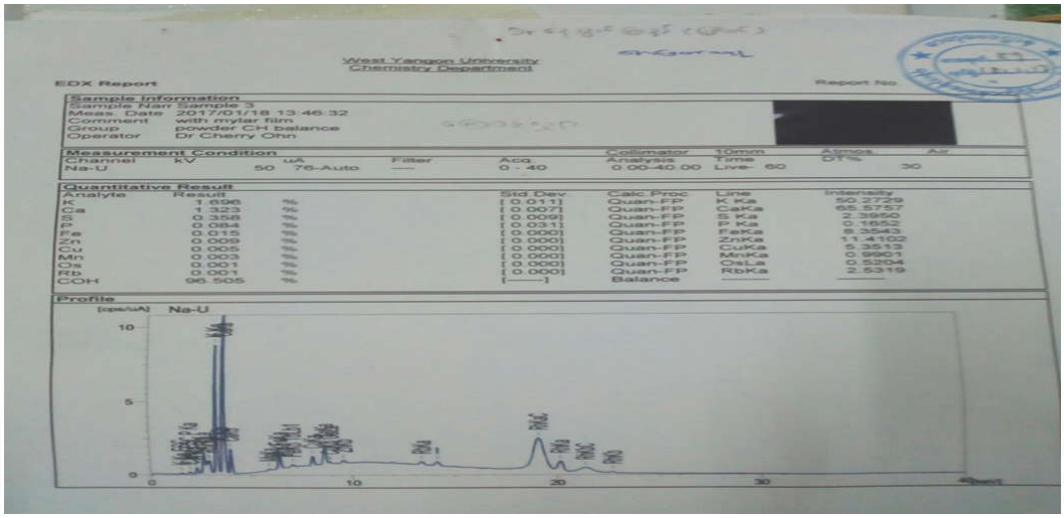

Figure 5. EDXRF spectrums of *Oroxylum indium* (L.)Vent (midnight horror) leaf

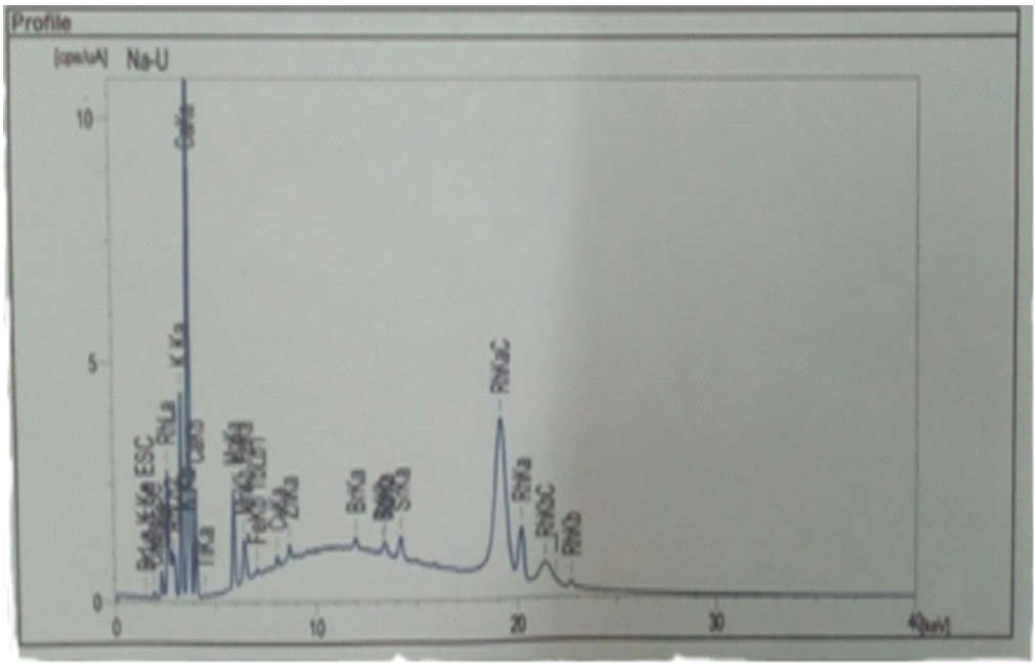

Figure 6. EDXRF spectrums of *Melastoma melabathricum* L. (Malabar) leaf

Table 1. Summary of the Phytochemical Investigation Results of Three Selected Leaves

No	Tests	Henna Leaves	midnight horror Leaves	Malabar Leaves
1.	Alkaloids	+	+	+
2.	Glycosides	+	+	+
3.	Flavonoids	+	_	+
4.	Carbohydrates	+	+	+
5.	Phenolic Compounds	+	+	+
6.	-amino acids	+	+	+
7.	Saponins	+	+	+
8.	Starch	-	+	-
9.	Tannins	+	+	+
10.	Reducing sugar	+	+	+
11	coumarins	+	_	_
12	phlobatannins	+	+	+

Table 2 Element Content in *Lawsonia inermis L, Oroxylum indium (L.)Vent* and *Melastoma melabathricu* L.

Element	Quantitative Results (%)		
	***Lawsonia inermis* L.**	***Oroxylum indium* (L.)Vent**	***Melastoma melabathricu* L.**
Ca	1.071	1.696	1.605
K	1.034	1.323	0.642
S	0.335	0.358	0.461
P	0.051	0.084	ND
Fe	0.016	0.015	0.010
Mn	0. .005	0.003	0.036
Br	0. 002	ND	0.001
Zn	0. 002	0.009	0.002
Sr	0. 002	ND	0.002
Cu	0. 002	0.005	0.001
Rb	0.000	0.001	0.001
Os	ND	0.001	ND
Ti	ND	ND	0.003
COH	97.481	96.505	97.038

N D =Non detective

Table 3. Inhibition Zone (mm) of Antimicrobial Activity for Aqueous Extract from Leaves of *Lawsonia inermis* L. *Oroxylum indium* (L.)Vent and *Melastoma melabathricum* L.

Compound	Zone of inhibition (mm)					
	Gram- positive bacteria			Gram- negative bacteria		Fungi
	B. Sub	*S.aureus*	*B.pumils*	*Pseudomonas*	*E.coli*	*Candida*
Lawsonia inermis L.	14mm (+)	15mm (++)	14mm (+)	13mm (+)	13mm (+)	13mm (+)
Oroxylum indium(L.)Vent	13mm (+)	13mm (+)	13mm (+)	11mm(+)	12mm (+)	12mm (+)
Melastoma melabathricum L.	13mm (+)	12mm (+)	12mm (+)	12mm (+)	13mm (+)	12mm (+)

Agar well – 10mm *Organisms*

10mm ~ 14mm (+) *Bacillus sublilis* (N.C.T.C-8236), *Bacillus pumilus* (N.C.I.B- 8982)
15mm ~ 19mm (++) *Staphylococcus aureus* (N.C.P.C-6371), *Candida albican*
20mm above (+++) *Pseudomonas aeruginosa* (6749) , *E-coli* (N.C.I.B -8134)

The present amount of elements present in *Lawsonia inermis* L, *Oroxylum indium* (L.)Vent and *Melastoma melabathricum* L. was shown in Table 2 and figure 4,5 and 6.The antimicrobial activity of the aqueous extracts of three different leaves were also determined by Agar well diffusion method The antibacterial and antifungal activities of aqueous extract from leaves, such as *Lawsonia inermis* L. *Oroxylum indium* (L.)Vent and *Melastoma melabathricum* L.tested against the following microorganism like *Staphylococcus aureus, Bacillus pumilus* and *Bacillus subtilis* as gram-positive bacteria, *Pseudomonas aeruginosa* and *Escherichia coli* as gram-negative bacteria and *Candida albicans* as fungal strain by Agar-diffusion method. The results indicated that *Lawsonia inermis* L.has shown the maximum antibacterial and antifungal activities against all tested microorganism as shown in Table 3 and figure 7.

The *Lawsonia inermis* L. showed maximum zone of inhibition against *Staphylococcus aureus*, (15 mm) followed by *Bacillus subtilis* (14 mm), *Bacillus pumilus* (14 mm), *Pseudomonas aeruginosa* (13 mm), *Escherichia coli* (13mm) and *Candida albicans* (13 mm). The *Oroxylum indium* (L.)Vent showed maximum zone of inhibition against *Staphylococcus aureus*, (13 mm) followed by *Bacillus subtilis* (13 mm), *Bacillus pumilus* (13 mm), *Pseudomonas aeruginosa* (11 mm), *Escherichia coli* (12mm) and *Candida albicans* (12 mm). *Melastoma melabathricum* L. showed maximum zone of inhibition against *Staphylococcus aureus*, (12mm) followed by *Bacillus subtilis* (13 mm), *Bacillus pumilus* (12 mm), *Pseudomonas aeruginosa* (12 mm), *Escherichia coli* (13mm) and *Candida albicans* (12 mm). The overall observation of antimicrobial activity of aqueous extract from three leaves indicated that the *Lawsonia inermis* L. have more impact than

the *Oroxylum indium* (L.)Vent and *Melastoma melabathricum* L. This is due to the presence of coumarins in Lawsonia inermis. Coumarins have many pharmacological activities such as anticlotting, hypotensive, antimicrobial, anti-inflammatory, and antitumor activities .Among the *Oroxylum indium* (L.)Vent and *Melastoma melabathricum* L. *Oroxylum indium* (L.)Vent has shown the moderate against the gram- positive bacteria than the *Melastoma melabathricum* L.

Conclusion

From the result it can be concluded that the aqueous extract from leaves of *Lawsonia inermis* L.,*Oroxylum indium* (L.)Vent and *Melastoma melabathricum* L. showed the presence of many phytochemical constituents which are responsible for antimicrobial property. Preliminary phytochemical profiling reflects essential information regarding the diversity of different classes of secondary metabolites such as alkaloids, flavonoids, steroids, saponins, tannins, reducing sugars etc. in the plant extracts. In the present study, qualitative tests for all three crude extracts showed significant indication about the presence of various secondary metabolites. Among the three selected samples, aqueous extract from leaves of *Lawsonia inermis* L. has shown the maximum antibacterial and antifungal activities against all tested microorganism than aqueous extract from leaves of *Oroxylum indium* (L.)Vent and *Melastoma melabathricum* L.. From the result data, aqueous extract from leaves of *Oroxylum indium* (L.)Vent showed more zone of inhibition towards gram positive bacterial strain than gram negative bacteria and fungi. The aqueous extract from leaves of *Melastoma melabathricum* L. showed more zone of inhibition towards *Bacillus subtilis* and *Escherichia coli* bacteria strain. Therefore, it suggests that the aqueous extract from leaves of *Lawsonia inermis* L. *Oroxylum indium* (L.)Vent and *Melastoma melabathricum* L. can be a source of traditional medicine to be used i

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