ANTIBACTERIAL PLANT EXTRACTS AND FORMULATION OF MOUTHWASH

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Abstract

In this study, we examined the medicinal properties of medicinal plants *Achyranthes aspera* Linn and *Jatropha gossypifolia* individually as well as their synergism. Different solvents system used to extract the principle compound present in these selected plants and solvent were chosen based on their polarity from high polar solvent to low polar. The ethanol extract of *Achyranthes aspera* and *Jatropha gossypifolia* has maximum activity against the microorganism *Staphylococcus aureus*. The effective compounds present in the extracted sample were evaluated by generating chromatogram in Thin Layer Chromatography technique. The quality and purity were confirmed by HPLC. The synergistic activity of ethanolic extract of these plants shows significant antibacterial activity.

Keywords: Thin Layer Chromatography, HPLC, *Achyranthes aspera*, *Jatropha gossypifolia*

Introduction:

A various types of plants having medicinal activities and the plants which are used in herbalism are called as Medicinal plants. Besides the medicinal plants plays a critical role to eradicate many infectious disease around the world. Dental caries and periodontal have been considered as the important global health disorders. There are many of chemically derived drugs to treat dental caries but they have side effects. In this study, actvities of Achyranthes aspera and Jatropha gossypifolia were studied.

Achyranthes aspera: *Achyranthes aspera linn* is a popular plant which has the activity against dental caries (Abhijit Dey, 2011). This plant also possess some pharmacological activity such as analgesics, antipyretic activities. It is used to treat many problems such as piles, digestive disorders, asthma, fever, cough, dysentery, psoriasis, paralysis, spleen enlargement, control of fertility, and postpartum bleeding (Charles Lekhya Priya, et al 2010). The parts of this plant is also used in the treatment of urinary tract infection, upper respiratory tract infection and for some sexually transmitted diseases caused bacteria and fungi (Lakshmi Naidu, et al 2006). *Achyranthes aspera* contains the phytochemical constituents such as saponin, sterols, polysaccharides and alkaloids (Charles Lekhya Priya, et al 2010). *Achyranthes aspera* is also used as a medicine for snake bites and their leaves were used for hydrophobia (Mohinder Kaur, et al).

Jatropha gossypifolia: *Jatropha gossypifolia* have antimicrobial activity (Rajesh Gaikwad, et al 2012).*Jatropha gossypifolia* contains an phytochemical constituents alkaloids and the stem contains ligan and other constituents present in this plant are saponin, tannin, phenolic compounds, flavonoid, curcin, triterpenes, diterpene, jatrophone, jatropholones A and B, jatrophatrione, apigenin, and cyclogossine A (Apurba Sarker Apu, et al 2013). *Jatropha gossypifolia* leaves contain jatropholone, naringenin, histamine, apigenin, vitexin, isovitexin and tannins (Saishri, et al 2015). *Jatropha gossypifolia* have antimicrobial, antioxidant activity, analgesic, and antidiarrheal activity. Especially the fruits of Jatropha gossypifolia have analgesic and antidiarrheal activity (Apurba Sarker Apu, et al 2013).

In this study, the antimicrobial activity of mixed plant extract was studied, and then the compounds present in this plant extract were identified. Traditionally these plants were used for the treatment of tooth decay. So, we also studied the combined effect of this plants against the organisms which plays a major role in the tooth decay. The mixed plants shows high inhibition against these organisms rather than a single plant extract. The ethanol extract from these plants contains variety of compounds which were identified by running thin layer chromatography. The serial extracts of *Achyranthes aspera* herb components has been investigated for in vitro antimicrobial activity against coccus aureus, hay bacillus, escherichia and Salmonella typhosa by disc diffusion methodology (Roma Yadav, et al 2016;Lakshmi Naidu, et al 2006).*A.aspera* possess high antioxidant activity and can used for the isolation of antioxidant compounds. (Charles lekhya priya, et al 2010).A qualitative phytochemical analysis was carried out and found to possess bioactive compounds like alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins. (Abi Beaulah, et al 2011).This review also includes reports onmorphology, anatomy, ecology, plant pathology, tissue culture, chromosome study and medicinal uses of the plant. (AbhijitDey, 2011; MohinderKaur, et al 2005).Hence endophytes are serving as alternative sources of drug molecules (Jyoti goutam, et al 2017).Degree of variation of antifungal and antibacterial activity of different parts of Jatropha sp. was observed. (Rajesh Gaikwad, et al 2012).The findings of the study clearly indicate the presence of significant analgesic, neuropharmacological and anti-diarrheal properties of the plant, which demands further investigation including, compound isolation (ApurbaSarkerApu, et al 2013) Products derived from plants may potentially control microbial growth in diverse situations and in the specific case of disease treatment, numerous studies have aimed to describe the chemical composition of those plant antimicrobials and therefore the mechanisms concerned in microbic growth inhibition, either separately or associated with conventional antimicrobials (Singh, 2015).Among those traditional plants, we have, Centellaasiatica, Euclea, Euphorbia, Foeniculum vulgare, Tulbaghia violacea, from 7 different plant families in our study (ManikandanDhayalan, et al 2015; Saishri, et al 2017).This review aims to provide an up to date overview of the phytochemistry, ethnomedicinal and pharmacological activities of J. gossypiifolia while providing an insight for future research towards both ethno pharmacological validation of its popular uses and its exploration as a new source of herbal medicinal products (OmololaTemitope Fatokun, et al 2016). Consequently, additional biological investigations essential to be carried out isolated compounds present in this plant (Sachin Jain, et al 2017). *A.aspera* treatment iatrogenic expression of a selected macromolecule of relative molecular mass thirty one.5 kD in both diabetic and immune compromised wound models, signifying that this particular protein might play a key role in A.aspera mediated wound healing in these two models (Barua, et al 2010).Toxicity of both the tested materials against Channapunctatus was time and dose dependent. But in case of Musmusculus, the toxicity was only dose dependant and the LC doses was much higher in comparison to fish Channapunctatus (Pratibha Singh, et al 2012).Themethanolic extract treated rats showed high diuretic effect as compared to control but this effect was less than furosemide. Significant increase in renal clearance of sodium, potassium and chloride ions was observed in treated and standard groups (Saurabh Srivastav, et al 2011).The developed and validated method can be used as quality control tool for the analysis of extract and herbal formulations containing Achyranthes aspera using betaine as marker (Tatke, et al 2014).Due to rich source of phytochemicals, this plant is may be used for herbal medicine (Veena Sharma, et al 2013).The combined in vitro and in vivo Anti-inflammatory screening shows that the ethyl acetate fraction of the crude extract of Valerian awallichii and Achyranthes aspera Linn can be used for the isolation of new Anti-inflammatory lead compounds (FazliKhuda, et al 2013).protein, glycosides, alkaloids, tannins and phenolic compound, steroid reducing sugars and saponin glycosides. These observations can facilitate within the Pharmacognostical identification and standardization of the drug within the crude kind and additionally to differentiate the drug from its adulteration (Dhale, et al 2013).

Materials and Methods:

The whole plant of *Achyranthes aspera* and *Jatropha gossypifolia* were used for this study. These plants were collected from the surrounding area of sevalpatti, sivakasi, virudhunagar (dt).

This powder form of sample was soaked in the solvents as the ratio of 10g per 100ml of solvent for five days.Then the sample extract was filtered out using whatman filter paper and stored in refrigerator for future use. The p^H values of the samples were identified as 6 for Ethanol extract, Petroleum ether extract and mixed extracts of *Achyranthes aspera* and *Jatropha gossypifolia*. The p^H value is 7 for water extract of *Achyranthes aspera* and *Jatropha gossypifolia* and 6 for mixed water extract.

Zone of inhibition

The antibacterial activity of plant extract was identified by the formation of zone against the microorganisms such as *Staphylococcus aureus, Escherichia coli,* and *Lactobacillus acidophilus.* The organisms were isolated and cultured in their respective media. For culturing of *Staphylococcus aureus* MS media is used and for *Lactobacillus acidophilus*, MRS media is used and then for *E. coli*, Nutrient agar is used.

The antibacterial activity was identified by using agar well diffusion method.

High Performance Liquid Chromatography

This technique is used to separate and quantify the compounds in the sample. 30 µl of these extracts were passed through 45 µm syringe filter and then filtrate was used for HPLC analysis. The HPLC system (Shimadzu lab chromo 2010 HT HPLC, UV detector) was used. The software package used for analysing results was Shimadzu lab chromo HPLC control and auto sampling . Chromatographic analysis was carried out using a C -18 column at 35℃ temperature. Prior to analysis, the column was equilibrated with the corresponding. The running conditions included: Injection volume 15µl, Mobile phase: Aceto nitrile: water running time 25 minute, Flow rate 1 ml per minute and detection at 254 nm.

Formulation

Mouth wash was formulated using mixed extract of plants which has the best activity against the microorganisms. The composition of mouthwash is listed below:

Ingredients	**Composition (%)**
Sodium fluoride	0.44
Mannitol syrup (70%)	15
Ethanol	10
Sodium lauryl sulphate	0.4
Sucrose	0.04
Mint oil (flavour)	0.15
Plant extract	Make up to 100 ml

The extracts based mouthwash were then subjected to microbial studies using certain pathogens. The Mannitol, Sucrose are act as sweeteners, and Mint oil act as flavour.

RESULTS AND DISCUSSION

Zone of inhibition

Based on the results, the plant extract has high activity against the microorganisms such as *S. aureus* and *E.coli*. When comparing all the samples of the plant extract, the mixed plant extract from ethanol shows high activity against both microorganisms.

Table 1: Antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Lactobacillus acidophilus* using agar well diffusion method at the concentration of 200µl.

Plant extract	***E. coli***	***S. aureus***	***L. acidophilus***
*Achyranthes aspera (*water*)*	1.5	0.3	0.3
*Achyranthes aspera (*pet. E*)*	1.2	1	0.3
*Achyranthes aspera (*E)	1	1	0.1
*J. gossypifolia (*water*)*	1.2	0.1	0.5
*J. gossypifolia (*Pet.E*)*	0.5	0.2	0.3
*J. gossypifolia (*E*)*	1	0.8	0.1
Mixed (water)	0.2	0.1	0.3
Mixed (Pet. E)	0.5	0.2	-
Mixed (E)	1.3	1	-
Antibiotics: Ampicillin	1.2	0	0.2
Antibiotic: Tetracycline	1.2	1.5	1

Thin layer chromatography

There are many compounds were identified by using thin layer chromatography technique. The different solvents were used to isolate the compounds present in the sample. By analysing the results the ethanol extracts of plants gives the best result. In this study the silica gel is used as stationary phase and calcium sulphate is used as a binding agent. Many solvents does not separate the compounds. The compound separation was based on the polarity of the mobile phase solvent. The compounds identified were tabulated below:

Table 2: Solvent: Methanol

Plant extract	Compounds	R_f value
Achyranthes aspera (W)	NO	0
Achyranthes aspera (E)	Pheophytin,chlorophyll b, carotenoids	0.888
Achyranthes aspera(PE)	Chlorophyll	0.540
J. gossypifolia (w)	Flavonoids	0.604
J. gossypifolia (E)	Flavonoids	0.520
J. gossypifolia (PE)	Flavonoids, chlorophyll	0.760
Mixed (W)	Chlorophyll	0.55
Mixed (E)	No	0
Mixed (PE)	Flavonoids, Chlorophyll	0.782

By using this solvent, ethanol extract of *Achyranthes aspera* and Petroleum ether extract of *Jatropha gossypifolia* has high R_f value, and More than two compounds were identified in this extract.

Table 3: Solvent: Petroleum ether

Plant extract	Compounds	R_f value
A.aspera (W)	NO	0
A.aspera (E)	Pheophytin,chlorophyll b	0.178
A.aspera(PE)	No	0
J. gossypifolia (w)	Carotenoids	0.3
J. gossypifolia (E)	Flavonoids, chlorophyll	0.355
J. gossypifolia (PE)	No	0
Mixed (W)	No	0
Mixed (E)	Chlorophyll, carotenoids	0.444
Mixed (PE)	Chlorophyll	0.096

By using this solvent as mobile phase the ethanol extract of combined plants has high R_f value and two compounds were identified.

Table 4: Solvent: Mixer of methanol and petroleum ether(1:1)

Plant extract	Compounds	R_f value
A.aspera (W)	Chlorophyll, flavonoids	0.442
A.aspera (E)	Pheophytin,chlorophyll b	0.825
A.aspera(PE)	No	0
J. gossypifolia (w)	Carotenoids, flavonoids	0.740
J. gossypifolia (E)	Flavonoids, chlorophyll	0.75
J. gossypifolia (PE)	Chlorophyll	0.277
Mixed (W)	Lutein, chlorophyll	0.657
Mixed (E)	Chlorophyll	0.325
Mixed (PE)	Lutein	0.116

By using this solvent as mobile phase Ethanol extract of Achyranthes aspera has high R_f value and two compounds were identified.

Table 5: Solvent: Ethanol: water (1:3)

Plant extract	Compounds	R_f value
A.aspera (E)	Pheophytin,chlorophyll	0.5277
J. gossypifolia (E)	Flavonoids, chlorophyll, carotenoids	0.615
Mixed (E)	Chlorophyll, carotenoids, lutein	0.914

There is no other compounds identified in the other plant extract when using this solvent as mobile phase. But ethanol extract of combined plants extracts has high R_f value and three compounds were identified.

Table 6: solvent: Ethanol: Water (1:4)

Plant extract	Compounds	R_f value
A.aspera (E)	Flavonoids, lutein	0.26
J. gossypifolia (E)	Chlorophyll, carotenoids	0.55
Mixed (E)	Pheophytin, lutein	0.312

The water and petroleum ether extract do not give any results while using this solvent as mobile phase. The Ethanol extract of Jatropha gossypifolia has high R_f value and two compounds were identified.

Table 7: Solvent: Ethanol: Water (1:9)

Plant extract	Compounds	R_f value
A.aspera (E)	Flavonoids, carotenoids	0.319
J. gossypifolia (E)	Chlorophyll	0.55
Mixed (E)	Pheophytin, lutein	0.25

By using this solvent as mobile phase Ethanol extract of Jatropha gossypifolia has high R_f value and compounds were identified.

Table 8: Solvent: Methanol: chloroform: Acetic acid (1:18:1)

Plant extract	Compounds	R_f value
A.aspera (E)	Flavonoids,pheophytin	1
J. gossypifolia (E)	Chlorophyll, carotenoids	1
Mixed (E)	Lutein	0.77
A.aspera(PE)	Chlorophyll, carotenoids, leutin	1
J. gossypifolia (PE)	Pheophytin, carotenoids, chlorophyll	1
Mixed (PE)	Chlorophyll	0.428

By using this solvent as mobile phase petroleum ether extract of Jatropha gossypifolia has high R_f value and three compounds were identified.

Table 9: Solvent: Butyl alcohol: Acetic acid: Water (4:1:5)

Plant extract	Compounds	R_f value
A.aspera (E)	Flavonoids	1
*J. gossypifolia (*E*)*	Chlorophyll, carotenoids	0.9
Mixed (E)	Pheophytin, lutein	1
A.aspera(PE)	Chlorophyll	1
J. gossypifolia (PE)	Flavonoids, chlorophyll	0.689
Mixed (PE)	Chlorophyll	1

By using this solvent as mobile phase petroleum ether extract has good R_f value and two compounds were identified.

Table 10: Solvent: Methanol: chloroform (1:9)

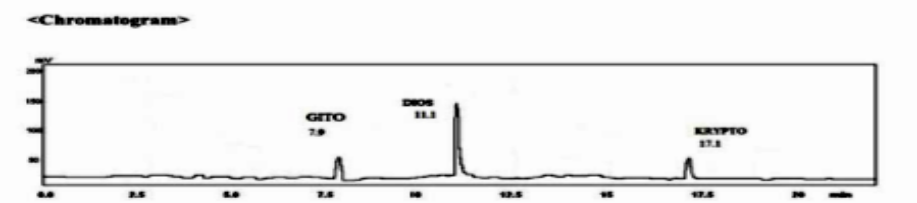
Plant extract	Compounds	R_f value
A.aspera (E)	Chlorophyll, flavonoids, Terrein (reddish spot)	0.66
J. gossypifolia (E)	Chlorophyll	0.895
Mixed (E)	Pheophytin, slutein, chlorophyll, Terrein	0.95

By using this solvent as mobile phase ethanol extract of combined plant extract has high R_f value and three compounds were identified.

High Performance Liquid Chromatography

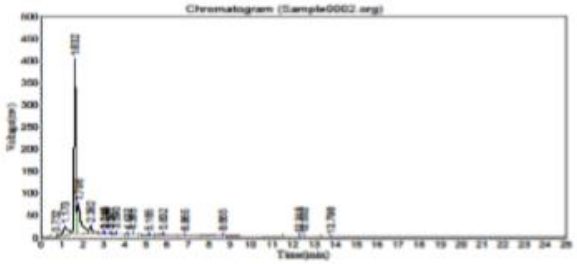
The compounds identified in the plant extract were separated, identified and quantified by using HPLC technique. The Retention time varies for each compounds. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used. The amount of retardation depends on the nature of the analyte and composition of both stationary and mobile phase. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time.

Figure 1: HPLC result of *Achyrathes aspera*



Peak	Name	Retention Time (min.)	Area(mm^2)	Conc.($\mu g\ g^{-1}$)
1.	Gitogenin (GITO)	7.9	5.06	4.11 ±0.58
2.	Diosgenin (DIOS)	11.1	7.38	6.32 ±0.20
3.	Kryptogenin (KRYPTO)	17.1	4.88	3.54 ±0.12

Figure2: HPLC result of *Jatropha gossypifolia.*

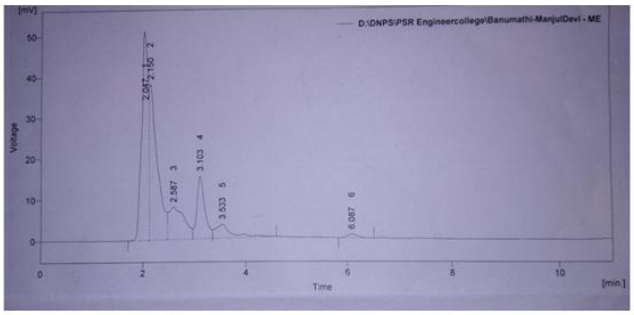


Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		0.732	53.308	197.810	0.0016
2		1.173	16096.449	299684.781	8.4104
3		1.632	397299.063	2233997.250	62.6097
4		1.798	84803.461	830190.825	23.3003
5		2.382	13153.874	127304.219	3.5729
6		3.015	436.207	2189.354	0.0614
7		3.088	719.966	6237.131	0.1751
8		3.307	853.882	5113.019	0.1435
9		3.390	1247.046	4180.000	0.1173
10		4.122	97.706	609.017	0.0171
11		4.365	552.000	8049.870	0.2259
12		5.165	79.364	166.500	0.0047

Figure 3: HPLC result Mixed Ethanol extract

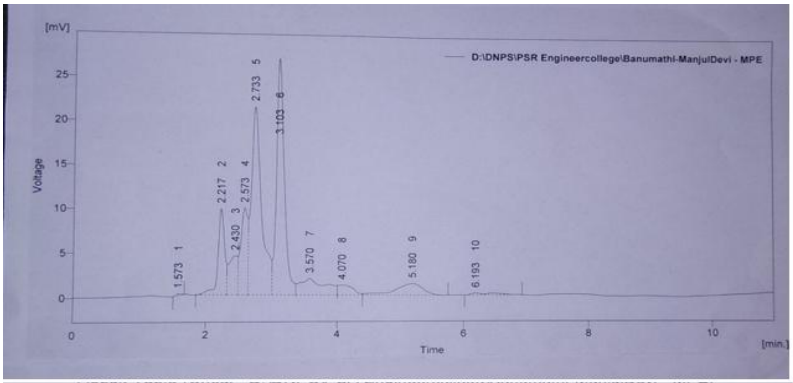
The peaks in the graph represent the compounds present in the plant extract. These compounds were identified based on the retention time. The retention time ranges between 8to13 represents presence of flavonoids.



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.047	456.848	51.015	34.6	43.2	0.15
2	2.150	409.426	38.228	31.0	32.4	0.15
3	2.587	170.714	7.895	12.9	6.7	0.38
4	3.103	163.066	15.355	12.4	13.0	0.15
5	3.533	74.185	3.386	5.6	2.9	0.30
6	6.087	12.395	0.901	0.9	0.8	0.21
7	13.640	33.564	1.322	2.5	1.1	0.36
	Total	1320.199	118.101	100.0	100.0	

Figure 4: HPLC result for Mixed Petroleum Ether extract

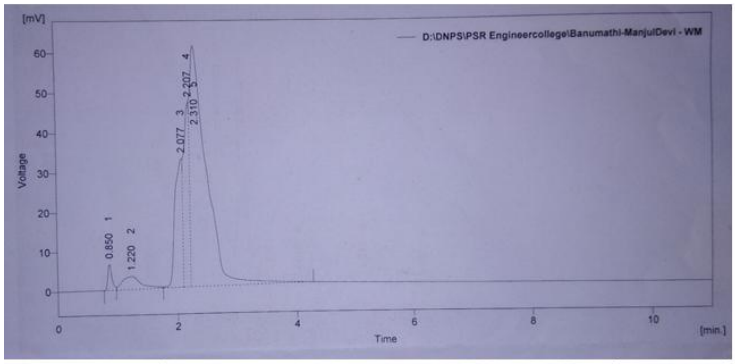
There are ten compounds present in this sample.



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.573	0.801	0.192	0.1	0.2	0.12
2	2.217	67.111	9.703	9.0	12.6	0.11
3	2.430	39.861	4.370	5.3	5.7	0.17
4	2.573	71.332	9.798	9.5	12.8	0.13
5	2.733	233.335	21.383	31.2	27.8	0.16
6	3.103	223.257	26.902	29.9	35.0	0.12
7	3.570	49.686	1.825	6.6	2.4	0.64
8	4.070	17.798	1.095	2.4	1.4	0.28
9	5.180	38.384	1.299	5.1	1.7	0.44
10	6.193	5.782	0.222	0.8	0.3	0.18
	Total	747.349	76.786	100.0	100.0	

Figure 15: Mixed Water for HPLC

The compounds present in the Achyranthes aspera and Jatropha gossypiifolia from ethanol extract was analysed based on the analysis, we determine both plant extract from ethanol has same components as well as in the synergism.



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.850	31.562	6.594	1.7	4.4	0.07
2	1.220	72.185	3.273	4.0	2.2	0.32
3	2.077	284.035	32.906	15.5	21.8	0.15
4	2.207	288.014	46.921	15.8	31.1	0.12
5	2.310	1150.995	61.159	63.0	40.5	0.28
	Total	1826.791	150.852	100.0	100.0	

Formulation of mouth wash

Plant extract	***E.coli***	***S.aureus***	***L.acidophillus***
Mixed ethanol	1	1.2	1.4

From the mixed ethanol extract, we observed more acivity against the microorganisms.

conclusion

Thus there are many compounds present in the mixed plant extract of *Achyranthes aspera* and *Jatropha gossypifoli*a and then they shows a good activity against bacteria. The ethanol extract of plants shows the best result than other solvents. Thus these plants can also be used for dental caries problem in future. These plants also be used for the treatment of wound healing, asthma and diarrhoea.

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