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Research Article

Antibacterial activity assessment of petroleum ether and methanolic extracts of *Achyranthes aspera* Linn (Amaranthaceae)

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Abstract
Achyranthus aspera is a common weed and known for various medicinal properties. The aim of the present study was to evaluate the antibacterial activities of different concentrations of methanolic and petroleum-ether leaf extracts of *A. aspera* against three gram-positive bacteria (*Micrococcus luteus, Bacillus subtilis, Streptococcus mitis*) and six gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi, Salmonella paratyphi A (MTCC-3220), Shigella flexneri*). The phytochemical screening of the leaf extract of the herb indicated the presence of flavonoides, tannins, saponins, polyphenolic compounds, alkaloids and glycosides. The methanolic extract at the highest concentration of 10 mg/ml showed prominent antibacterial activity in two gram-negative bacteria, i.e. *K. pneumoniae* and *E. coli* with 22 mm zone of inhibition and one gram-positive bacterium i.e. *M. luteus* with 19 mm zone of inhibition. The methanolic extract at 0.0781mg/ml concentration showed least antibacterial activity against all tested bacteria produced a zone of inhibition between 10 to 12 mm while petroleum ether extract of same concentration had moderate antibacterial activity against *S. flexneri* (15 mm zone of inhibition). It can be concluded that novel compounds like flavonoids, tannins, saponins, alkaloid, and polyphenolic compounds in *A. aspera* leaves have potent antimicrobial property.

Keywords: *Achyranthus aspera,* Antibacterial activity, Gram-negative, Gram-positive, Phytochemical

INTRODUCTION

The leading cause of World-wide deaths is due to infectious diseases. Though pharmaceutical industries have produced a wide range of antibiotics, resistance to these drugs by bacteria has increased as they have the genetic ability to acquire resistance. These drug-resistant bacteria are more pathogenic with high mortality rate and become a great challenge in the pharmaceutical and healthcare industry (Westh *et al*., 2004). To overcome these antibiotic-resistant bacteria, researchers are looking for alternative and novel drugs. According to the World Health Organization (WHO), medicinal plants would be the best natural source to obtain a variety of compounds for the treatment of various infectious diseases (Vijayan *et al*., 2007). About 80% of the world's population relies on traditional medicines which has compounds derived from medicinal plants. In comparison to synthetic antibiotics, plant based drugs cause less or no side effects (Burt, 2004; Shariff *et al*., 2006; Dubey *et al*., 2011).
Phytotherapy, the treatment of disease by the use of plants is a very old practice when a primitive man out of necessity and by intuition began to use plants to

overcome his sufferings from injuries and diseases. The plants that possess substances which can be used for therapeutic purposes or can be used for the synthesis of a drug are called medicinal plants (Rishikesh, *et al*., 2013) In recent years, several studies have been conducted to prove the significance of antimicrobial properties of plant extracts and phytochemicals in therapeutic treatments (Rates, 2001; Gordon and David, 2005; Kumar *et al*., 2010; Singh *et al*., 2013). Plants have an extraordinary ability to synthesize compounds, i.e. secondary metabolites like terpenoids, quinines, flavonoids, tannins etc. that are responsible for protecting plants from microorganisms, insects and other natural pests (Selvamohon *et al*., 2012; Singh *et al*., 2013; Hasan, 2014). Plants accumulate these secondary metabolites in all cells, but their concentration varies in different parts. The leaf is the part where the highest accumulation of these bioactive compounds takes place. In the recent past, there has been a tremendous increase in the use of these plant products for producing antibacterial drugs in developing as well as developed countries.

World Health Organization (WHO) has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species (Srivastav *et al*., 2011). Out of these, only a small number has been systematically investigated for their antimicrobial activities (Sharma *et al*., 2009). One of the plants is *Achyranthus aspera* which has been reported to contain many phytochemicals (Tiwary *et al*., 2018) but there is a dearth of studies on the antibacterial action of leaves of this plant.

The investigators observed that the plant *A. aspera* has several medicinal properties. But not much work has been reported so far on the *in vitro* antimicrobial activities of methanolic and petroleum ether extract of leaves of this weed. Hence, the present study was made to assess the antibacterial activity of the leaves of *A. aspera* so that it can be used for human welfare.

MATERIALS AND METHODS

Plant material: *A. aspera* Linn (Amaranthaceae) commonly known as Chirchita in Hindi, an annual stiff, erect herb is found as a weed throughout India. It is about 1-2 meter in height, often with a woody base having an angular or ribbed stem, simple or branched from the base with thick, ovate-elliptic or obovate-rounded leaves. The flowers are greenish-white, and seeds are reddish-brown in colour. The plants were collected from the campus of the Regional Institute of Education, Bhubaneswar and taxonomically identified by the Department of Botany, Utkal University, Bhubaneswar, Odisha.

Preparation of plant extract: The fresh leaves of the plant were harvested in the month of August and washed properly with distilled water so as to remove dust and other foreign particles. The leaves were shade dried at room temperature in the laboratory with proper ventilation and ambient temperature. The air-dried leaves were further dried in a hot air oven at 40^0C for 24 hours to remove moisture content. The completely dried leaves were ground into powder using a mechanical grinder. Then the dried powdered material was stored in airtight bottles. 60 gram of pulverized leaves were placed in Soxhlet extraction unit and exhaustively defatted with petroleum ether (60-80°) for 40-45 hours. To confirm whether the extraction is complete or not, the extract from the siphon tube of soxhlet is taken in a watch glass. If no residue remained in the watch glass, the extraction was complete, if not the extraction continued. The extracted plant material was then air-dried, repacked in soxhlet apparatus and then successively and thoroughly extracted with methanol for 40-45 hours. The crude extract obtained was filtered and distilled to evaporate the solvent from the extract. The liquid extract was concentrated separately under vacuum, and the resulting dried extract was preserved in a desiccator until further use.

The dried petroleum ether and methanolic extracts were weighed, and the percentage yield was calculated by the formula mentioned below (Truong *et al*., 2019):

Percent yield of extract = weight of dried extract X 100/ weight of dried plant material ……..Eq. 1

Percent yield of petroleum ether extract= 6.334/160 X 100 = 3.96% w/w ……..Eq. 2

Percent yield of methanolic extract= 6.31/160 X 100 = 10.19% w/w ……..Eq. 3

Colour of petroleum ether extract was yellowish-green while the colour of methanolic extract: was greenish-black. The percentage yield of methanolic extract, i.e. 10.19%w/w was more than petroleum ether extract, i.e. 3.96%.

Preliminary phytochemical analysis: Preliminary phytochemical screening was performed by following the methodology of Harborne *et al*. (1998). To check the presence or absence of primary and secondary metabolites, all the extract were subjected to a battery of chemical tests. The extracts of *A. aspera* were analyzed for the presence of carbohydrates, protein, alkaloids, saponins, tannins, triterpenes, cardiac glycoside, anthraquinones, steroid, coumarin and flavonoids by following standard methods (Williamson *et al*., 1996; Banso and Ngbede, 2006; Ngbede *et al*., 2008). The different chemical tests performed for phytochemical screening were: (i) for carbohydrates - Fehling's test and Molisch's test; (ii) for cardiac glycosides - Baljet test and Keller-killiani test (for deoxy sugars); (iii) for steroids and triterpenoids - Raymond's test (for lactose

ring), Liebermann-burchard test, Hansch's test and Seliwanoff's test; for proteins - Millon's test, Xantho-proteic test and Biuret test; (iv) for alkaloids - Dragendorff's test, Mayer's test, Hager's test and Wagner's test; (v) for glucosides - Keller-killiani test, Baljet test and Legal test; (vi) for saponins - Foam test; (vii) for tanins - Gelatin-Lead Acetate test; (viii) for phenolic compounds - Ferric chloride test; (ix) for flavonoids - Shinoda test and (x) for terpenoids - Liebermann-burchard test.

Antibacterial activity assay

Test microorganisms used: The *in vitro* screening for antibacterial activity was carried out using few selected Gastrointestinal, urinary tract and skin infections causing pathogens which included three gram-positive bacteria *(Micrococcus luteus, Bacillus subtilis, Streptococcus mitis)* and six gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi, Salmonella paratyphi A (MTCC-3220), Shigella flexneri).* These organisms were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. These organisms were identified by standard microbiological methods (Murray *et al*., 1995). The organisms to be tested were inoculated into sterile nutrient agar. After an incubation period of 24 hr at 37°C, a loop of inoculums was transferred into 5 ml of nutrient broth and incubated for 2 hr at 37°C which served as fresh suspension inoculums.

Determination of antibacterial activity: In the present study, two methods, i.e. (1) Agar well-diffusion method and (2) MIC value determination, were carried out to study the antibacterial activity of *A. aspera.*

Agar well-diffusion method: Antibacterial activities of plant extracts were tested by cup diffusion method on nutrient agar medium (Satish *et al*., 1999). The culture plates were developed by pouring 20 ml of nutrient agar medium (Hi-Media) into sterile Petri plates. The inoculums suspension was spread uniformly over the agar medium using sterile cotton swabs to get uniform distribution of bacteria. Using a flamed borer, four wells (6 mm diameter) at the corner were made in the media at a distance of 2-3 cm from the periphery of the plates and then the wells were labelled. 50µl of each plant extract at different concentration were poured aseptically into the well. Standard antibiotic and blank (with the same solvent) were used as a positive and negative control, respectively. Then the plates were incubated for 24 hr at 37ºC to allow maximum growth of the microorganism. The potency of these extracts was recorded by measuring the diameter of zone of inhibition expressed in millimetre using an antibiotic scale, and the diameter of ≥10 mm was considered as inhibitor zone. Triplicates were performed, and the average value was recorded.

MIC value determination (Two-fold serial dilution): The minimum inhibitory concentration is defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates. The Minimum Inhibitory Concentration (MIC) of the petroleum ether and methanolic extracts of *A. aspera* against the test microorganism were determined by using serial dilution technique (Reiner, 1982). The MIC is to determine the lowest concentration of an antibacterial agent that appears to inhibit the growth of the bacteria. The density of the bacterial suspension was standardized by using McFarland standard method (McFarland, 1907). The methanolic extract was dissolved in dimethyl formamide or DMF (6%), this was previously tested for antibacterial activity against all test bacteria and found to have no antibacterial activity. The petroleum ether extract was solubilised in a mixture of dimethyl formamide and a surfactant SDS (sodium dodecyl sulphate-2%). A stock solution of 10mg/ml was prepared. Subsequently two-fold serial dilutions were made in concentration range of 5mg/ml, 0.125mg/ml, 0.3125mg/ml and 0.7812mg/ml. Well with DMF served as negative control and well with antibiotics dissolved in DMF served as a positive control. 50µl of diluted plant extract of different concentrations was added in the respective wells and 50µl of DMF for control. Gentamicin (0.01mg/well) and Ciprofloxacin (0.01mg/well) served as standard antibiotics against gram-negative bacteria while Ampicillin (10µg/disc) and Bacitracin (8units/disc) were used as standard antibiotic drug against gram-positive bacteria. The plates were incubated for 24 hours at 37°C. The MIC values of the extracts were determined by two-fold serial dilution assay.

RESULTS

Phytochemicals present in petroleum ether and methanolic extract: The preliminary analysis of petroleum ether and methanolic extracts of leaves of *A. aspera* revealed the presence of a number of

Table 1. Phytochemicals present in petroleum ether and methanolic extract of leaves of *A. aspera.*

Phytochemical compounds	Petroleum ether extract	Methanolic extract
Carbohydrates	-	+
Alkaloids	-	+
Glycosides	-	+
Tannins and polyphenolic compounds	+	+
Saponins	-	+
Flavonoids	+	+
Proteins and free amino acids	-	+
Steroids	+	+

Table 2. Antibacterial activity of petroleum ether and methanol extracts of *A. aspera* leaves at 10mg/ml concentration and standard antibiotics against some human pathogenic bacteria at 0.01mg/ml and 0.05mg/ml. (P- Petroleum ether extract, M- Methanolic extract, G- Gentamicin, Cf- Ciprofloxacin, C- Chloramphenicol, Cfx- Cefixime. NZI- No zone of inhibition, NT- Not tested).

Microorganisms (Bacteria)	Diameter of Zone of Inhibition (in millimeter)					
	Type of Plant Extract (mg/ml)		Standard Drugs (mg/disc)			
	P (10)	M (10)	G (0.10)	Cf (0.01)	C (0.01)	Cfx (0.005)
M. luteus (Gram+ve)	15±0.08	19±0.67	25.3±0.57	35.6±0.57	22.6±0.57	18.6±1.52
S. mitis (Gram+ve)	13±1.65	13±0.56	NT	NT	NZI	17±1.00
B. subtilis (Gram+ve)	13±1.87	17±0.45	NT	NT	NZI	NZI
S. paratyphi (Gram–ve)	15±00	16±0.45	20.6±0.57	39±0.00	NZI	18.6±0.57
E. coli (Gram–ve)	16±0.14	22±1.34	31±0.00	38±0.00	21±0.00	20.3±0.6
S. flexneri (Gram–ve)	22±0.78	14±1.05	22±0.00	35.6±0.57	17.6±0.6	14.6±1.52
P. aeruginosa (Gram–ve)	12±0.00	14±0.15	22.6±0.57	34±1.00	NZI	NZI
K. pneumoniae (Gram–ve)	12±0.66	22±0.45	21±0.00	37±0.00	20±0.00	15.6±0.57
S. typhi (Gram–ve)	13±0.11	16±0.65	21±0.00	31±0.00	NZI	16.6±1.52

Table 3. Antibacterial activity of petroleum ether and methanol extracts of *A. aspera* leaves at 5mg/ml concentration and standard antibiotics against some human pathogenic bacteria at 0.01mg/ml and 0.05mg/ml. (P- Petroleum ether extract, M- Methanolic extract, G- Gentamicin, Cf- Ciprofloxacin, C- Chloramphenicol, Cfx- Cefixime. NZI- No zone of inhibition, NT- Not tested).

Microorganisms (bacteria)	Diameter of Zone of Inhibition (in millimetre)					
	Type of Plant Extract (mg/ml)		Standard Drugs (mg/disc)			
	P (5)	M (5)	G (0.01)	Cf (0.01)	C (0.01)	Cfx (0.05)
M. luteus (Gram+ve)	13±0.00	17±0.89	25.3±0.57	35.6±0.57	22.6±0.57	18.6±1.52
S. mitis (Gram+ve)	13±0.45	12±1.34	NT	NT	NZI	17±1.00
B. subtilis (Gram+ve)	12±0.00	14±0.34	NT	NT	NZI	NZI
S . paratyphi (Gram–ve)	14±0.34	19±0.65	20.6±0.57	39±0.00	NZI	18.6±0.57
E. coli (Gram–ve)	12±0.09	18±0.56	31±0.00	38±0.00	21±0.00	20.3±0.6
S. flexneri (Gram–ve)	18±0.67	15±0.56	22±0.00	35.6±0.57	17.6±0.6	14.6±1.52
P. aeruginosa (Gram–ve)	11±0.45	12±0.34	22.6±0.57	34±1.00	NZI	NZI
K. pneumoniae (Gram–ve)	12±0.87	21±0.67	21±0.00	37±0.00	20±0.00	15.6±0.57
S. typhi (Gram–ve)	12±0.15	13±1.87	21±0.00	31±0.00	NZI	16.6±1.52

Table 4. Antibacterial activity of petroleum ether and methanol extracts of *A. aspera* leaves at 1.25mg/ml concentration and standard antibiotics against some human pathogenic bacteria at 1.25µg/ml. (P- Petroleum ether extract, M- Methanolic extract, G- Gentamicin, Cf- Ciprofloxacin, C- Chloramphenicol, Cfx- Cefixime. NZI- No zone of inhibition, NT- Not tested).

Microorganisms (bacteria)	Diameter of Zone of Inhibition (in millimetre)					
	Type of Plant Extract (mg/ml)		Standard Drugs (mg/disc)			
	P (1.25)	M (1.25)	G (0.01)	Cf (0.01)	C (0.01)	Cfx (0.05)
M. luteus (Gram+ve)	12±0.09	15±0.07	25.3±0.57	35.6±0.57	22.6±0.57	18.6±1.52
S. mitis (Gram+ve)	10±1.54	11±0.67	NT	NT	NZI	17±1.00
B. subtilis (Gram+ve)	11±0.45	13±0.24	NT	NT	NZI	NZI
S. paratyphi (Gram–ve)	12±0.78	19±0.45	20.6±0.57	39±0.00	NZI	18.6±0.57
E. coli (Gram–ve)	11±1.07	16±1.67	31±0.00	38±0.00	21±0.00	20.3±0.6
S. flexneri (Gram–ve)	16±0.55	15±1.01	22±0.00	35.6±0.57	17.6±0.6	14.6±1.52
P. aeruginosa (Gram–ve)	10±0.00	11±0.00	22.6±0.57	34±1.00	NZI	NZI
K. pneumoniae (Gram–ve)	11±0.76	17±0.13	21±0.00	37±0.00	20±0.00	15.6±0.57
S. typhi (Gram–ve)	11±0.88	12±0.52	21±0.00	31±0.00	NZI	16.6±1.52

Micrococcus luteus ***Bacillus subtilis*** ***Streptococcus mitis***

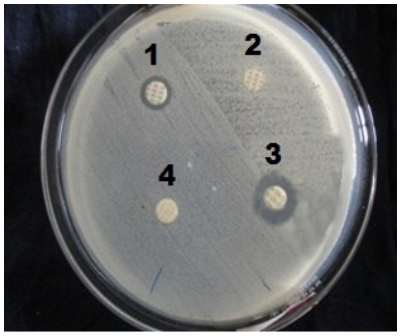

Fig. 1: *Antibacterial activity of different standard antibiotics.*

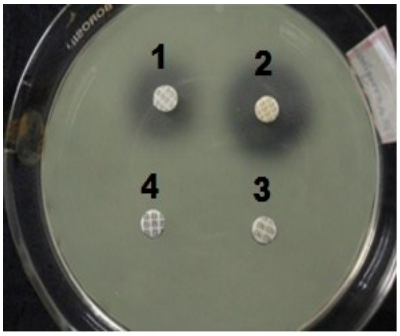

Fig. 2: *Antibacterial activity of different standard antibiotics.*

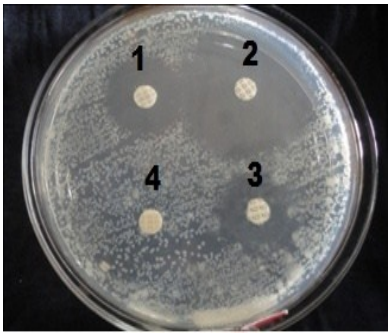

Fig. 3:*Antibacterial activity of different standard antibiotics.*

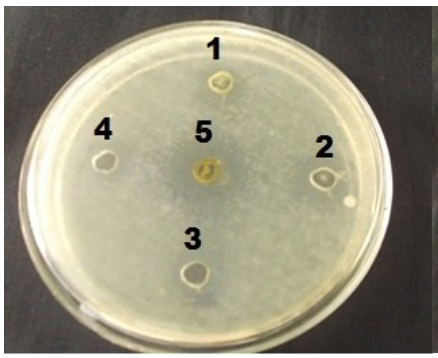

Fig. 4: *Antibacterial activity of methanolic extract.*

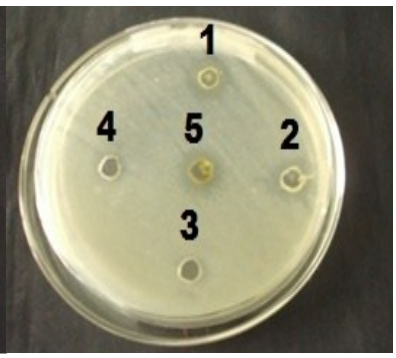

Fig. 5*: Antibacterial activity of methanolic extract.*

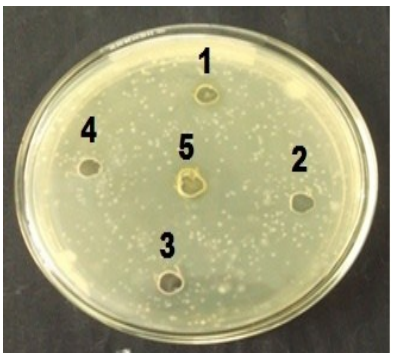

Fig. 6: *Antibacterial activity of methanolic extract*.

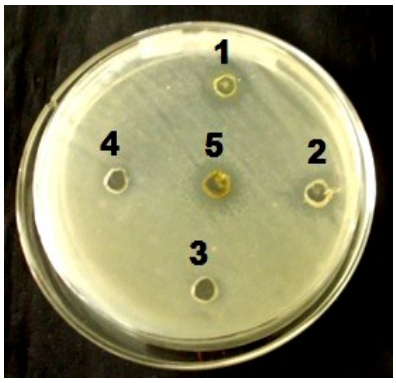

Fig. 7: *Antibacterial activity of petroleum ether extract.*

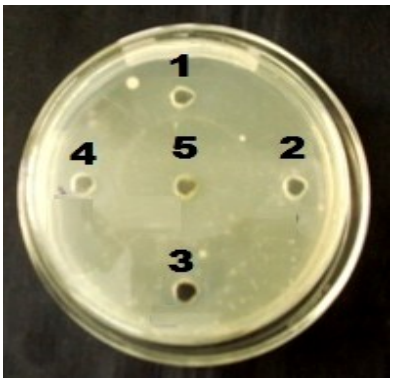

Fig. 8: *Antibacterial activity of petroleum ether extract.*

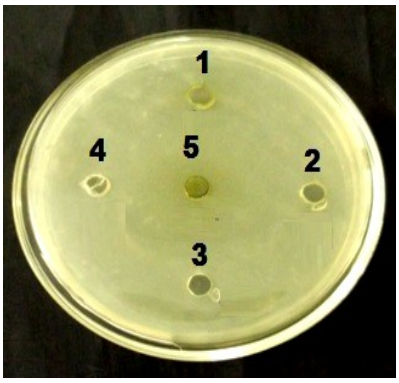

Fig. 9: *Antibacterial activity of petroleum ether extract.*

Plate 1: *Antibacterial activity of standard antibiotics, methanolic and petroleum ether extract against gram positive bacteria;* ***Fig.1 to 3.*** *Showing antibacterial activity of standard antibiotics (1) Ampicillin (10µg/disc) (2) Bacitracin (8units/disc), (3) Gentamicin (10µg/disc) and (4) Ciprofloxacin at 5µg/disc;* ***Fig. 4 to 6.*** *Showing antibacterial activity of methanolic extract.* ***Fig. 7 to 9****. Showing antibacterial activity of petroleum ether extract at the concentration of (1) 500µg/ml (2) 125µg/ml (3) 31.25µg/ml and (4) 7.812µg/ml (5)1000 µg/ml.*

phytochemicals as given in Table 1.

The petroleum ether and methanolic leaf extracts of *A. aspera* at different concentration showed antibacterial activity against nine different bacteria (*M. luteus, B. subtilis, S. mitis, E. coli, P. aeruginosa, K. pneumonia, S. typhi, S. paratyphi A (MTCC-3220), S. flexneri*).

Antibacterial activity of petroleum ether and methanolic extracts: The methanolic extract at the highest concentration of 10 mg/ml showed prominent antibacterial activity in two gram-negative bacteria, i.e. *K. pneumoniae* and *E. coli* with zone of inhibition 22mm while *M. luteus,* the gram-positive bacterium showed maximum zone of inhibition i.e. 19mm. It was moderately active against three gram-negative bacteria i.e. *S. flexneri, P. aeruginosa* and *S. paratyphi* with zone of inhibition ranging from 14-16mm and one gram-positive bacterium, i.e. *B. subtilis* showed zone of inhibition of 17mm. The methanolic extract showed the least antibacterial activity against *S. mitis* with a minimum zone of inhibition of 13mm. On the other

Table 5. Antibacterial activity of petroleum ether and methanol extracts of *A. aspera* leaves at 0.3125mg/ml concentration and standard antibiotics against some human pathogenic bacteria at 0.3125µg/ml. (P- Petroleum ether extract, M- Methanolic extract, G- Gentamicin, Cf- Ciprofloxacin, C- Chloramphenicol, Cfx- Cefixime. NZI- No zone of inhibition, NT- Not tested).

Microorganisms (bacteria)	Diameter of Zone of Inhibition (in millimetre)					
	Type of Plant Extract (mg/ml)		Standard Drugs (mg/disc)			
	P (0.3125)	M (0.31205)	G (0.01)	Cf (0.01)	C (0.01)	Cfx (0.05)
M. luteus (Gram+ve)	13±0.09	11±1.67	25.3±0.57	35.6±0.57	22.6±0.57	18.6±1.52
S. mitis (Gram+ve)	10±0.78	11±0.56	NT	NT	NZI	17±1.00
B. subtilis (Gram+ve)	11±0.23	12±1.09	NT	NT	NZI	NZI
S. paratyphi (Gram–ve)	12±0.78	12±1.56	20.6±0.57	39±0.00	NZI	18.6±0.57
E. coli (Gram–ve)	10±1.45	11±0.34	31±0.00	38±0.00	21±0.00	20.3±0.6
S. flexneri (Gram–ve)	17±1.00	13±0.67	22±0.00	35.6±0.57	17.6±0.6	14.6±1.52
P. aeruginosa (Gram–ve)	11±0.55	10±1.05	22.6±0.57	34±1.00	NZI	NZI
K. pneumoniae (Gram–ve)	11±0.56	17±0.36	21±0.00	37±0.00	20±0.00	15.6±0.57
S. typhi (Gram–ve)	10±0.88	12±0.67	21±0.00	31±0.00	NZI	16.6±1.52

Table 6. Antibacterial activity of petroleum ether and methanol extracts of *A. aspera* leaves at 0.0781mg/ml concentration and standard antibiotics against some human pathogenic bacteria at 0.0781µg/ml. (P- Petroleum ether extract, M- Methanolic extract, G- Gentamicin, Cf- Ciprofloxacin, C- Chloramphenicol, Cfx- Cefixime. NZI- No zone of inhibition, NT- Not tested).

Microorganisms (bacteria)	Diameter of Zone of Inhibition (in millimetre)					
	Type of Plant Extract (mg/ml)		Standard Drugs (mg/disc)			
	P (0.0781)	M (0.0781)	G (0.01)	Cf (0.01)	C (0.01)	Cfx (0.05)
M. luteus (Gram+ve)	11±0.067	10±1.05	25.3±0.57	35.6±0.57	22.6±0.57	18.6±1.52
S. mitis (Gram+ve)	10±0.15	12±0.76	NT	NT	NZI	17±1.00
B. subtilis (Gram+ve)	10±1.05	10±0.54	NT	NT	NZI	NZI
S. paratyphi (Gram–ve)	11±1.25	12±0.05	20.6±0.57	39±0.00	NZI	18.6±0.57
E. coli (Gram–ve)	10±0.34	12±0.04	31±0.00	38±0.00	21±0.00	20.3±0.6
S. flexneri (Gram–ve)	15±0.57	10±0.07	22±0.00	35.6±0.57	17.6±0.6	14.6±1.52
P. aeruginosa (Gram–ve)	10±0.11	10±0.67	22.6±0.57	34±1.00	NZI	NZI
K. pneumoniae (Gram–ve)	10±0.98	12±0.45	21±0.00	37±0.00	20±0.00	15.6±0.57
S. typhi (Gram–ve)	12±0.11	12±1.05	21±0.00	31±0.00	NZI	16.6±1.52

Table 7. MIC values of Petroleum ether and Methanolic extract of *A. aspera* plant against bacteria tested by serial dilution assay.

Sl. no.	Microorganisms (Bacteria)	Petroleum ether extract MIC (mg/ml)	Methanolic extract MIC (mg/ml)
1	*Micrococcus luteus*	0.0781	0.3125
2	*Streptococcus mitis*	5	0.3125
3	*Bacillus subtilis*	0.3125	0.0781
4	*Pseudomonas aeruginosa*	0.3125	0.3125
5	*Klebsiella pneumoniae*	0.3125	0.0781
6	*Shigella flexneri*	0.0781	0.0781
7	*Salmonella typhi*	1.25	0.0781
8	*Salmonella paratyphi A*	1.25	0.0781
9	*Escherichia coli*	0.3125	0.0781

hand, the petroleum ether extract at the concentration of 10mg/ml was most effective against one gram-negative bacterium, i.e. *S. flexneri* while moderately effective against one gram-positive (*M. luteus*) and two gram-negative (*S. paratyphi* and *E. coli*) bacteria. The petroleum ether extract was least potent against *P. aeruginosa* and *K. pneumoniae* with minimum zone of inhibition of 12mm (Table 2; Plates 1-3).

The methanolic extract at the concentration of 5mg/ml amongst gram negative bacteria showed prominent antibacterial activity against *E. coli*, *K. pneumoniae,* and *S. paratyphi.* It was moderately active against one gram positive bacterium, i.e. *M. luteus,* and two gram-negative bacteria, i.e. *S. flexneri and B. subtilis.* The

Escherichia coli ***Pseudomonas aeruginosa*** ***Klebsiella pneumonia***

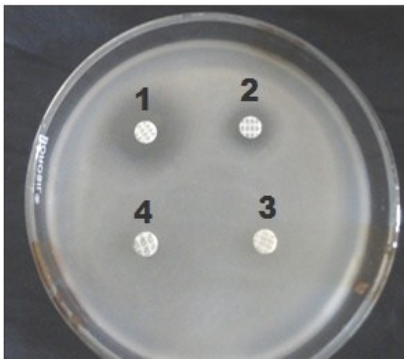

Fig. 10:*Antibacterial activity of different standard antibiotics.*

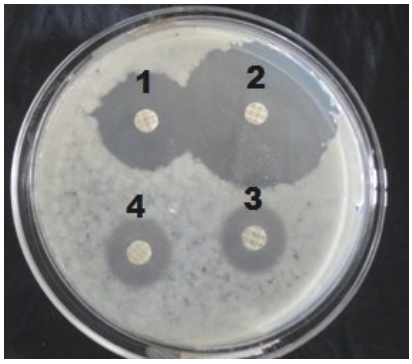

Fig. 11: Antibacterial activity of different standard antibiotics.

Fig. 12: Antibacterial activity of different standard antibiotics.

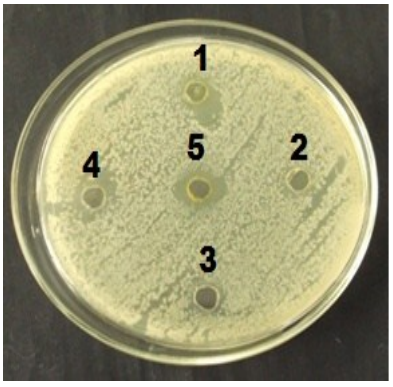

Fig. 13: *Antibacterial activity of methanolic extract.*

Fig. 14: *Antibacterial activity of methanolic extract.*

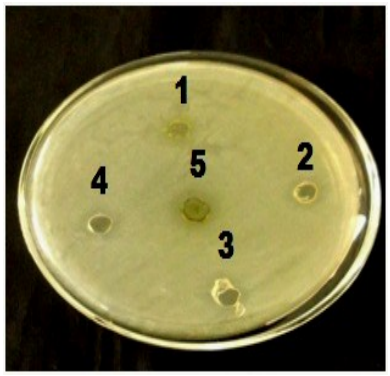

Fig. 15: *Antibacterial activity of methanolic extract.*

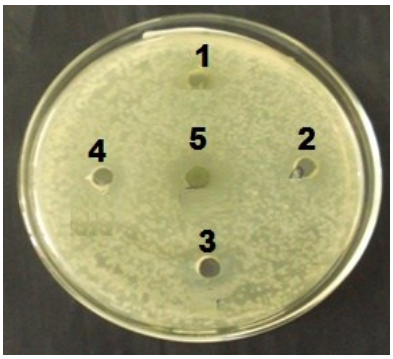

Fig. 16: *Antibacterial activity of petroleum ether extract.*

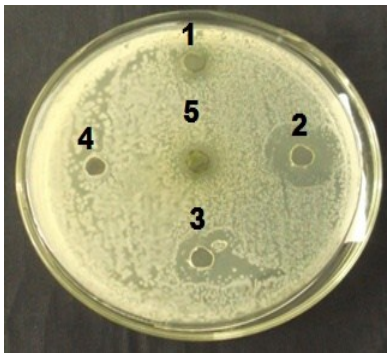

Fig. 17: *Antibacterial activity of petroleum ether extract.*

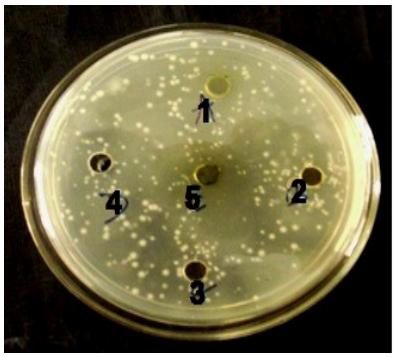

Fig. 18: *Antibacterial activity of petroleum ether extract.*

Plate 2: *Antibacterial activity of standard antibiotics, methanolic and petroleum ether extract against gram negative bacteria;* **Fig. 10 to 12**. *Showing antibacterial activity of standard antibiotics (1) Ampicillin (10µg/disc) (2) Bacitracin (8units/disc), (3) Gentamicin (10µg/disc) and (4) Ciprofloxacin at 5µg/disc;* **Fig. 13 to 15**. *Showing antibacterial activity of methanolic extract and Fig. 16 to 18. Showing antibacterial activity of of petroleum ether extract at the concentration of (1) 500µg/ml (2) 125µg/ml (3) 31.25µg/ml and (4) 7.812µg/ml (5)1000 µg/ml.*

extract was least effective against *S. mitis* and *P. aeruginosa.* On the other hand petroleum ether extract at the diluted concentration show prominent activity against *S. flexneri* but was moderately active against all gram-negative and gram-positive bacteria except for *P. aeruginosa, E. coli and B. subtilis* with 11-12mm as the diameter of zone of inhibition (Table 3; Plates 1-3).

The methanolic extract at the concentration of 1.25mg/ml showed maximum antibacterial activity against *S. paratyphi* moderate activity against *E. coli, S. flexneri* and *K. pneumococci* (Table – 4). The extract was least potent against *S. mitis and P. aeruginosa.* The petroleum ether extract was maximum effective against *S. flexneri* while least effective against *S. mitis* and *P. aeruginosa* **(**Plates- 1 to 3).

The methanolic extract at 0.3125mg/ml concentration showed prominent antibacterial activity against *K. pneumonia* and showed least effect against *P. aeruginosa*. The petroleum ether extract at same concen-

Salmonella typhi

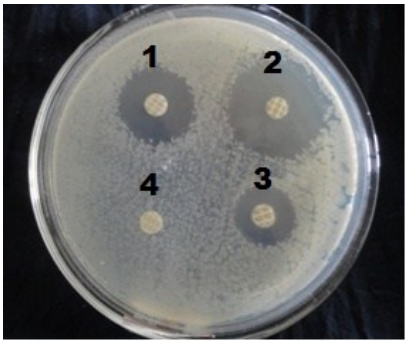

Fig. 19: *Antibacterial activity of different standard antibiotics.*

Salmonella paratyphi A

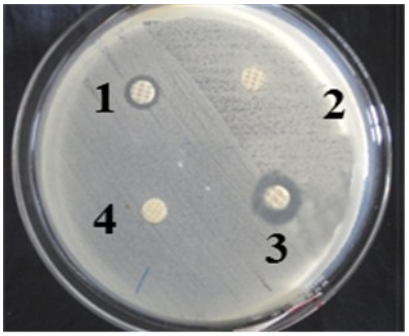

Fig. 20: *Antibacterial activity of different standard antibiotics.*

Shigella flexneri

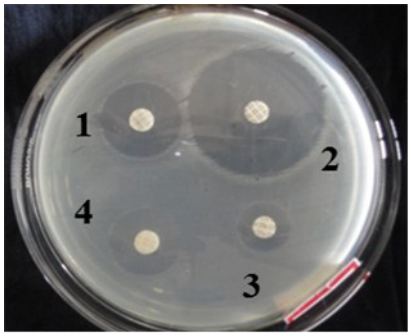

Fig. 21: *Antibacterial activity of different standard antibiotics.*

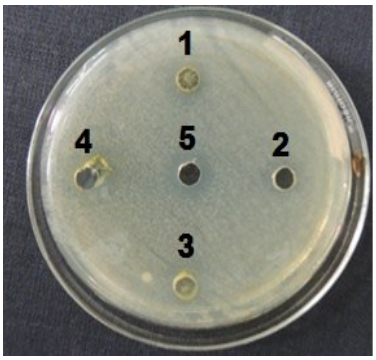

Fig. 22: *Antibacterial activity of methanolic extract.*

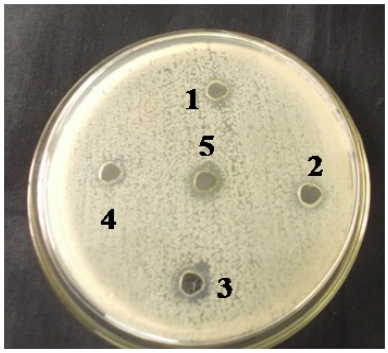

Fig. 23: *Antibacterial activity of methanolic extract.*

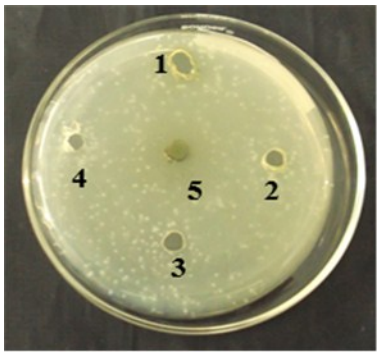

Fig. 24: *Antibacterial activity of methanolic extract*.

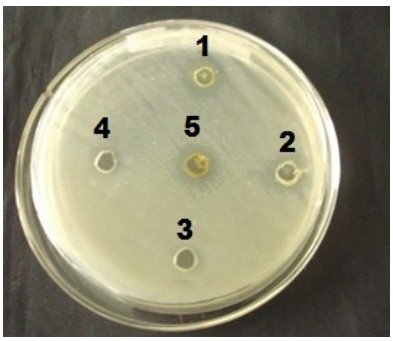

Fig. 25: *Antibacterial activity of petroleum ether extract*.

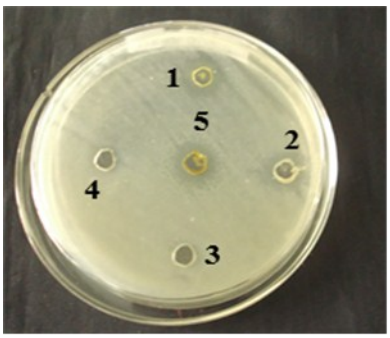

Fig. 26: *Antibacterial activity of petroleum ether extract.*

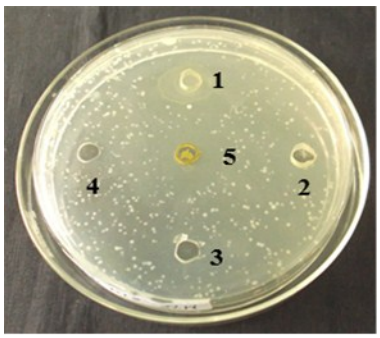

Fig. 27: *Antibacterial activity of petroleum ether extract*.

Plate 3: *Antibacterial activity of standard antibiotics, methanolic and petroleum ether extract against gram negative bacteria;* **Fig. 19 to 21.** *Showing antibacterial activity of standard antibiotics (1) Ampicillin (10µg/disc), (2) Bacitracin (8units/disc), (3) Gentamicin (10µg/disc) and (4) Ciprofloxacin at 5µg/disc;* **Fig. 22 to 24.** *Showing antibacterial activity of methanolic extract and Fig. 25 to 27. Showing antibacterial activity of of petroleum ether extract at the concentration of (1) 500 µg/ml (2) 125µg/ml (3) 31.25µg/ml and (4) 7.812µg/ml (5)1000 µg/ml.*

tration had maximum effect on *S. flexneri* and least effect on *E. coli* and *S. mitis.* The rest bacterial strain showed almost no zone of inhibition indicating its resistance against the extract at 0.3125 mg/ml concentration (Table 5; Plates 1-3).

The methanolic extract at 0.0781mg/ml concentration showed least antibacterial activity against all tested bacteria while petroleum ether extract of same concentration had moderate antibacterial activity against *S. flexneri* (Table – 6). The rest bacterial strain shows almost no zone of inhibition, indicating its resistance against the extract at that concentration (Plates 1-3).

Comparative pictorial representations of potency of petroleum ether and methanolic leaf extract of *A. aspera* at different concentrations with that of standard drugs against the tested bacterial strains are shown in Plates- 1 to 3.

Determination of Minimum inhibitory concentration (MIC) against selected bacterial strains: The MIC value of methanolic extract was found to be low

compared to petroleum-ether extract. The methanolic extract was found to have low MIC values of 0.0781mg/ml for most of the bacteria except for *P. aeruginosa, S. mitis,* and *M. luteus* in which the MIC value is 0.3125mg/ml. With petroleum ether extract *S. mitis* showed a higher MIC value of 5mg/ml. The lower MIC value is an indication of high effectiveness of the extract, whereas higher MIC indicates the less effectiveness of the extract. The MIC values for different bacteria are given in Table 7.

DISCUSSION

The search for novel phytochemicals from natural sources has received much attention and efforts have been put in to identify compounds with the potential to act against antibiotic-resistant bacteria and also to replace synthetic ones. Several researchers have shown that plants are the natural sources of novel compounds like phenols, polyphenols, terpenoids, flavonoids etc that serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganisms like Doss *et al*. (2011) in *Medicago sativa*; Dubey *et al*. (2011) in *Ziziphus Mauritiana*; Mazid *et al*. (2011) in *Rauwolfia canescens*; Selvamohon *et al*. (2012) in *Aloe vera*, *Phyllanthus emblica*, *Cynodon dactylon*; Bagde *et al*. (2013) in *R. serpentina*; Iqbal *et al*., (2013) and Rohela *et al*. (2016) in *R. tetraphylla*. The majority of the active components in *A. aspera* are C-glycosyl flavones based on apigenin and luteolin; Harman alkaloids present in trace amounts along with sucrose and trace amounts of volatile oil (Leung and Froster, 1996; Newall *et al*., 1996). The major phytoconstituents of this plant are alkaloids, phenols, glycoside, flavonoids, and cyanogenic compounds, maltol, phytosterols, passifloricins, polyketides, and alphapyrones (Dhawan *et al*., 2004; Tiwari, *et al*., 2018). In the present study, the methanolic and petroleum ether leaf extract of *A. aspera* revealed the presence of phytochemicals like flavonoids, steroids, Tannins and polyphenolic compounds etc. (Table 1).

Isolation of phytochemical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Tiwary *et al*. (2018), in their study, showed that tannin was present in shoot and root extracts of *A. aspera* but not in the leaf extract. However, the phytochemical analysis of both methanolic and petroleum ether leaf extract of *A. aspera* in the present study clearly showed the presence of tannins. Sravanthi *et al*. (2013) reported absence of alkaloids and saponins in the alcoholic extracts of leaves of *A. aspera* while the results of the present study and Bajaj *et al*. (2012) detected the presence of these phytochemicals in methanolic extracts.

The use of several extracts to analyze the efficacy of plants for antimicrobial activity has also been realized by several scientists in many plant species like *Adhatoda zeylanica* and *Trianthema decandra* (Geethalakshmi *et al*., 2010)*, Argemone mexicana* (Rahaman *et al*., 2011), *Tinospora cordifolia, Cassia fistula* (Upadhyay, 2011), *Ocimum sanctum* (Singh *et al*., 2013) and *Carica papaya* (Shubam *et al*., 2019). The traditional practitioners make use of water primarily as a solvent, but the results of the present study showed that organic solvents were certainly much better and powerful. Similar results that methanolic extract is more potent than aqueous extract have been reported by Singh *et al*. (2013) in *O. sanctum*. This may be due to the better solubility of the active components in organic solvents and flavonoids are least stable in water which is the primary polyphenolic compound in plants. We found in this study the leaf extract of the *A. aspera* weed by methanol provided more consistent antimicrobial activity compared to those extracted by petroleum ether.

Plant based products have been effectively proven for their utilization as source for antimicrobial compounds. For instance, methanol extracts of *A. ferox* and *W. somnifera* exhibited inhibitory activity against all the strains of *N. gonorrhea,* while only the methanol extract of *W. somnifera* was effective against *C. albicans* (Kambizi and Afolayan, 2008). In the present study, the petroleum-ether and methanolic extracts of leaves of *A. aspera* showed the antimicrobial activity against six gram-negative bacteria (*E. coli, P. aeruginosa, K. Pneumococci***.** *S. typhi, S. paratyphi, S. flexneri)* and three gram-positive bacteria *(B. subtilis, M. luteus, S. mitis* **(**Table 2 - 6, Plates- 1 to 3**)**. The methanolic extract showed prominent antibacterial activity against gram negative bacteria, *K. pneumococci* and *E. coli* with a maximum zone of inhibition (22 mm) while it was moderately active against three gram-negative bacteria;*, S. paratyphi, S. typhi* and *S. flexineri* with zone of inhibition 16 mm at 10 mg/ml concentration (Table – 2). The methanolic extract showed least antibacterial activity against *V. cholera* and *P. aeruginosa* with a zone of inhibition of 13-14 mm. Among gram-positive bacteria, *M. luteus* showed notable antibacterial activity. On the other hand, petroleum ether extract was most effective against *S. flexneri* while moderately effective against *S. paratyphi, E. coli* and *M. lutes* bacteria (Table – 2). The petroleum ether extract was least potent against *B. subtilis, P. aeruginosa* and *K. pneumococci* with a minimum zone of inhibition. The results of the study showed a difference in the effect of different organic solvent extracts for antimicrobial activity and this difference in the activity between different solvent extract is due to the difference between extract compounds in this two extracts. These finding of the

present study corroborates similar research findings of Abi Beaulah *et al*. (2011) in *A. aspera* and Singh *et al*. (2013) in *Ocimum sanctum*. They have shown similar antibacterial activity of hexane, chloroform, ethyl acetate and methanol leaf extracts on *S. aureus*, *E. coli* and *P. mirabilis.* The plant may be considered as a biosynthetic laboratory for a multitude of compounds like alkaloid, glycoside, volatile oils, tannins, saponins, flavonoids etc. These compounds are termed as secondary metabolites and are responsible for therapeutic effects.

Conclusion

The present study concluded that leaf extracts of *A. aspera* contained potential antimicrobial components like flavonoids, saponins, alkaloids and tannins that may be of great use for the development of pharmaceutical industries as a therapy against diabetes (as it possesses hypoglycemic and hypolipidemic activity), cancer and cardiovascular abnormalities (reduces blood pressure and heart rate). The methanol and petroleum ether extracts of *A. aspera* possessed significant inhibitory effect against tested pathogens. The results of the study support the folklore claim along with the development of new antimicrobial drugs from the plant.

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