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CHEMICAL COMPOSITION AND PHARMACOGNESTIC STUDY OF CRUDE PLANT EXTRACT OF *VERNONIA ELAEAGNIFOLIA*

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ABSTRACT

In the present investigation, a field experiments aimed at evaluating phytoconstituents, chemical composition, antioxidants and antimicrobial activity of *V. elaeagnifolia* leaf. The gas chromatography coupled to mass spectroscopy GC-MS, eight compound were determined, the major compounds identified in the crude plant extract were Phorbol12,13-dihexanolate(19.84%), Cyclopropal (3,4) benz (1,2,e) azulene- (16.55%), Mibemycin, b,13-choro-5-demethoxy-28-epoxy-5(hydroxyimino)-25-(1-methylethy)(15.12%).The timicrobial susceptibility results obtained in this work revealed that the extraction yield of the different extraction solvents used was correlated with the antimicrobial activity observed. Of the isolates tested, only 3(MEE and EAE) revealed susceptibly to the extracts, with DMSOE revealing the highest susceptibility. DPPH radical scavenging activity of *Vernonia* leaf extract with ascorbic acid as reference where the IC_{50} value for the *Vernonia* extract (IC_{50} = 3259 µg/ml) and IC_{50} value was said to be moderate as compared to standard (IC_{50}= 2782 µg/ml).

KEYWORDS: ***V. elaeagnifolia,*** **Antimicrobial, DPPH, GC-MS**

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INTRODUCTION

The pharmaceutical industries have become increasingly more receptive to the potential use of antimicrobials, antioxidants and other drugs derived from plants. Furthermore, the general public has become increasingly aware of the overuse and misuse of antibiotics, and is now very interested in alternative medicines such as "medicinal plants". It has been estimated that between 20-80% of the populations in many countries use botanical products, and consider them to be safe and effective [1]. *V. elaeagnifolia* belongs to family *Asteraceae*. It is commonly called as Toran vel, Curtain creeper. It is an extensive, perennial, woody, ornamental climber. Young stem, slender, pendulous, glabrous, 0.4-0.6 cm in thickness, becomes woody at maturity. The plant is frequently grown as an ornamental plant in houses and gardens, especially on fencing, compounds walls and buildings reaching up to 7-8 m in height. Flowers pinkish-purple colored in small auxiliary heads. Heads crowded terminally in paniculate cymes, 1.5 cm in diameter and has single types of florets. *Vernonia elaeagnifolia* DC was studied earlier for its traditional use as a leech repellent[2]. *Vernonia* species *viz., V. ambigua, V. amygdalina and V. cinerea* were evaluated *in vitro* for their efficacy against *Cercosporell apersica* and *Curvularia lunatus* isolates of groundnut leaf spot disease[3]. *Vernonia cinerea* leaf is an ingredient of 'agbo' infusion for treatment of malaria, snake bite and a remedy for pile[4]. *Vernonia amygdalina* most common and readily available species with a lot of medicinal values, for example in the treatment of dermatophitic diseases[5], antibacterial activities[6], hepato protective and antioxidant activities[7] and control of sapolegnisis disease of fish[8]. In the present investigation, a field experiments aimed at evaluating chemical composition, antioxidants and antimicrobial activity of *V. elaeagnifolia* leaf was carried out.

MATERIALS AND METHODS

(i) Collection of plant material

The fresh leaves of *V. elaeagnifolia* climber were collected from Botanical garden of A.C.S College campus Dist-Amravati (Maharashtra) The experimental site is located between coordinates 20.91° N, 77.75°E and an altitude of 342 m in foothills of Central India experiencing the subtropical climate during winter season in the month December 2013 and the Authentication of the plant was confirmed by botanist Prof. S.K Tippat, Department of Environment Science , Art, Commerce & Science College, Amravati.

(ii) Chemicals and microbial cultures

All the chemicals and standard antibiotics used in this work were purchase from Sigma Aldrich, Merck and Hi-media, Mumbai, India. The reference bacterial strains used in this study were obtained from the American Type Culture Collection (ATCC) and Microbial Type Culture Collection (MTCC) Institute of Microbial Technology, Chandigarh, India. They were selected from gram positive and gram negative bacteria to represents a broad spectrum of potential pathogens that pose significant threats in the medical field (Table 1).

(iii) Preparation of plant extract

The plant were dried over ambient temperature and the dried sample were grind properly and dried powder sample was extracted in Methanol at 65ºC, Chloroform 61ºC, Dimethyl sulphoxide 189ºC, Ethyl acetate 77ºC and Distilled water 100ºC by using soxhlet apparatus[9] and extracts were concentrated by gradually evaporating the respective solvent on hot water bath. The concentrated extract was collected in sterile bottles and kept in a cool and dark place prior to analysis.

Table 1
Reference microbial strains and their clinical implications.

Organism	Reference	Clinical implication
Gram positive bacteria		
Staphylococcus aureus	ATCC-33591	Staphylococcal infections[10].
		Superficial or localized infections [11].
		Osteomyelitis [12].
		Scalded skin syndrome[13].
Propionibacterium acnes	ATCC-1951	CNS shunt infections[14].
		Cardiovascular[15].
		Chronic pseudophakic, endophthalmitis [16].
Gram negative bacteria		
Escherichia coli	ATCC-14948	Cholecystitis [17].
		bacteremia [18].
		cholangitis, urinary tract infection (UTI), and traveler's diarrhea, and other clinical infections such as pneumonia and neonatal meningitis [19].
Pseudomonas aeruginosa	MTCC-424	Ventilator as so-pneumonia [20].
		Endocarditis [21].
		Respiratory tract infections [22].
Klebsiella pneumoniae	MTCC-4030	Cholangitis, UTI [23].
		Nosocomial disease and pneumonia [24].
Salmonella typhi	ATCC-25812	Typhoid fever, enteritis [25].

Phytochemical analysis (Qualitative analysis)

All the crude extracts of *V. elaeagnifolia* were diluted in their respective solvents and subjected for qualitative preliminary Phytochemical screening to identify the presence of the secondary metabolites according to the standard methods[26], from the intensity of the color inferred for the tests, they were rated for their presence [27].

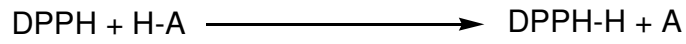
Antimicrobial Activity

Antimicrobial activity of five Organic extracts viz. aqueous, methanolic, chloroform, ethyl acetate and DMSO etc of *V. elaeagnifolia* leaves were determined by Agar disc diffusion assay according to the Manual of antimicrobial susceptibility testing [28], was used to assay the various antibiotics for bactericidal activity against test strains of *E. coli, K. pneumoniae, P. aeruginosa, S. aureus, P. acnes, S.typhi.* The strains of microorganisms obtained were inoculated in test tube containing 5 ml of nutrient broth. These test tubes were incubated at 37^0C for 24 h and were referred to as seed broth [29]. Media were prepared using Muller Hinton Agar poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter width had been impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37^0C. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Positive controls were used in experiments antibiotics (Tetracycline -10 mcg) as a standard.

Antioxidant activity of V. elaeagnifolia

The radical scavenging activity of the plant extracts against 2,2 Diphenyl -1-Picryl hydroxyl radical was determined by UV spectrophotometer carry 60 (Agilent). Antioxidant present in the plant usually quantified employing folins reagent i.e. DPPH is used as a quantify antioxidant. DPPH assay is often used to evaluate the ability of antioxidant to scavenge free radical which are known to be a major factor in biological damage to caused by oxidative stress [30].

Reaction:-Reduction of DPPH from stable free radical (purple). Antioxidant react with DPPH is often used to evaluate the ability of antioxidant to scavenge free radical which is known to be major factor which stable free radical become paired in the presence of H donor and reduce to DPPH-H to yellow colour.



Extract was examined by comparing it to activity of known antioxidant such as ascorbic acid by scavenging activity.

Antioxidant activity (DPPH free radical scavenging activity) of methanolic extract

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 2, 2-diphenyl-1- picrylhydrazyl (DPPH) free radical was determined by the method described by Shen [31]. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1000-5000 µg/ml solution. 3.94 mg of DPPH was prepared in 100 ml methanol and 2.96 ml of this solution was mixed with 40 µl of sample solution and standard solution separately. These solution mixtures were kept in dark for 20 min and optical density was measured at 517 nm using UV-Vis Spectrophotometer (Carry 60 - Agilent). DPPH solution was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below

% of DPPH Radical Scavenging activity = Abs control - Abs sample / Abs control ×100

Abs control is the absorbance of DPPH radical and methanol. Abs sample is the absorbance of DDPH radical + sample extract was the measure. Absorbance values were corrected for free radical decay using the blank solution. And IC_{50} values can calculate by using calibration curves verses percentage of inhibitions.

GC-MS Analysis of V. elaeagnifolia

1. Gas Chromatography

Gas Chromatography of the plant extract was carried out on a 6890 Gas Chromatography model 5765 equipped with direct injector and split ratio set to 10:1. (DB-5) (5% phenyl polysioxane, 30m length 250u internal diameter; 0.25um film coating) fused capillary column. Helium was the carrier gas at 1.0 ml min. The oven temperature program was programmed to start at 35 ^{o} hold for 2 min then temp at 20 ^{0}C per min to 300 ^{0} C and hold for 5 min. Injector and detector temperature were 220 ^{o}C and 230^{o}C respectively. Injection size was 0.02 ul neat.

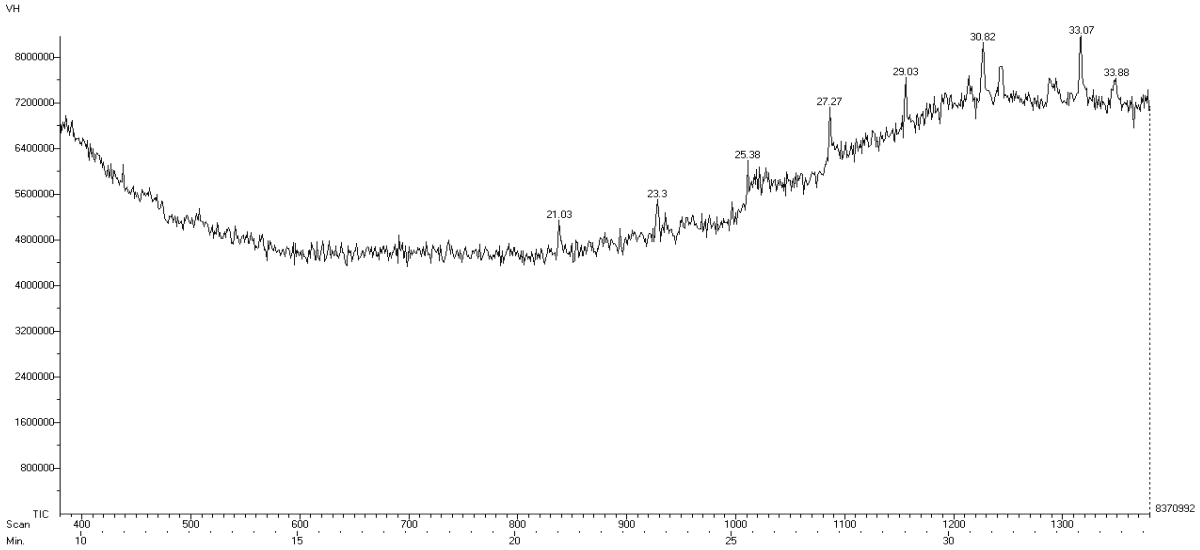


Figure 1
Gas Chromatogram of Leaves extract of Vernonia elaeagnifolia

2. Gas Chromatography and Mass Spectroscopy

A JEOL GC-mate II bench top double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-2000[1] software was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.

Identification of chemical constituents

Identification of the chemical constituents was done on the basis of retention index (RI) using a mass spectra library search NIST and by comparing the

mass spectral and retention data with literature [32]. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

RESULTS AND DISCUSSION

1. Phytochemical Analysis

Phytochemical evaluation is to confirm the presence of various chemical constituent present in plant. Screening of Phytochemical was done according to the polarity of five organic extract. Viz. aqueous, methanol, ethyl acetate, chloroform, DMSO. Phytochemical analysis listed in Table 2. Due to higher polarity of the DMSO extract show revealed presence of maximum phytochemical composition susceptibility as well as methanol and chloroform show in average amount of phytoconstituents and these phytoconstituents independently responsible for the broad range of medicinal properties.

Table 2
Phytochemical analysis of V. elaeagnifolia

S.N	Phytochemical	Tests performed	Aqueous extract	Methanolic extract	Chloroform extract	Ethyl acetate extract	DMSO extract
1	Steroid	Ring Test	-	-	-	-	-
2	Phytosterols	Liebermann Buchard Test	-	+	+	+	++
3	Tannins and Phenolic Compound	Ferric Chloride Test	-	-	-	-	-
		Lead Acetate Test	++	+++	-	+	++
		Gelatin Test	-	-	-	-	-
4	Terpenoids	Salkowski Test	+	+	-	++	++
5	Alkaloids	Mayer Test	-	+	-	-	+
		Dragendorff's Test	++	+++	+	++	+++
		Wagner Test	++	++	-	+	++
6	Flavonoids	Cynadine Test	+++	++	-	-	++
7	Cumarine	Fluorescence test	++	++	++	++	+++
8	Saponins	Foam Test	-	-	-	-	+

++ indicates: strong presence, + indicates: weak presence, - indicates: strong absence

2. Antimicrobial Activity

The result of antibacterial screening was carried out for five organic solvent plant extract (which already discussed in previous Para). Antibacterial activity of plant *V. elagenfolia* is listed in Table 3. The antibacterial screening is the major of the inhibition hollow observed in inhibition zone. The highest inhibition zone was observed in DMSO extract in each bacterial strain where E.coli shows mid active zone inhibition where *S.aureus* show less, the same observed in inhibition zone present study along with solvent methanol and ethyl acetate while aqueous and chloroform shows minimum inhibition zone in all bacterial strain viz. *S.typhi, P.acne, E.coli, P.aeru, K.pneu*. and for all isolates tested antibiotic was most efficient inhibition bacterial growth.

Table 3
Antimicrobial activity of Leaves Different solvents (20 µl) of V. elaeagnifolia

Organisms	Test Samples (Growth inhibition [a]) mm				Standard	
	AQE	MEE	CLE	EAE	DMSOE	AB
S.aureus	09±0.3	12.5±0.4	08±0.5	10.5±0.7	13±0.4	24 ±0.3
P.acne	10±0.1	13±0.3	12±0.4	11±0.4	15±0.6	27 ±0.4
E.coli	10.5±0.5	12±0.6	10±0.6	11±1	17 ±0.3	22 ±0.4
P.aeruginosa	08±0.2	11±0.2	08±0.6	10±0.6	13±0.4	23 ±0.2
K. pneumoniae	08±0.4	13±0.2	15±0.4	12±0.9	16 ±0.7	25±0.5
S.typhi	10.5±0.3	12±0.2	12±0.5	12±0.4	15±0.8	28 ±0.4

AQE-Aqueous extract, MEE-Methanolic extract, CLE-Chloroform extract, EAE-Ethyl acetate extract, DMSOE- Dimethyl sulphoxide extract, AB-Antibiotic, [a]- Values are represented as the mean ± S.D. of experiments.

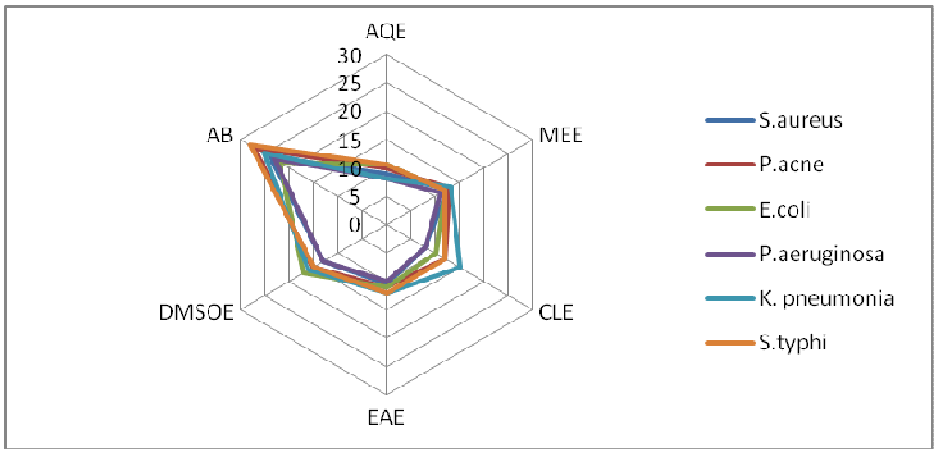


Figure 2
Mean zone of inhabitation off five organic extracts of V. elaeagnifolia

3. Antioxidant Activity

The radical scavenging activity of the *V. elaeagnifolia* leaf extract was tested using stable free radical DPPH (Deep purple colour) as, DPPH has the advantage of being unaffected by certain side reaction. Fig.3 show the DPPH radical scavenging activity of *Vernonia* extract with ascorbic acid as reference where the IC_{50} value for the *Vernonia* extract (IC_{50} = 3259 µg/ml) and IC_{50} value was said to be moderate as compared to standard (IC_{50}= 2782 µg/ml).

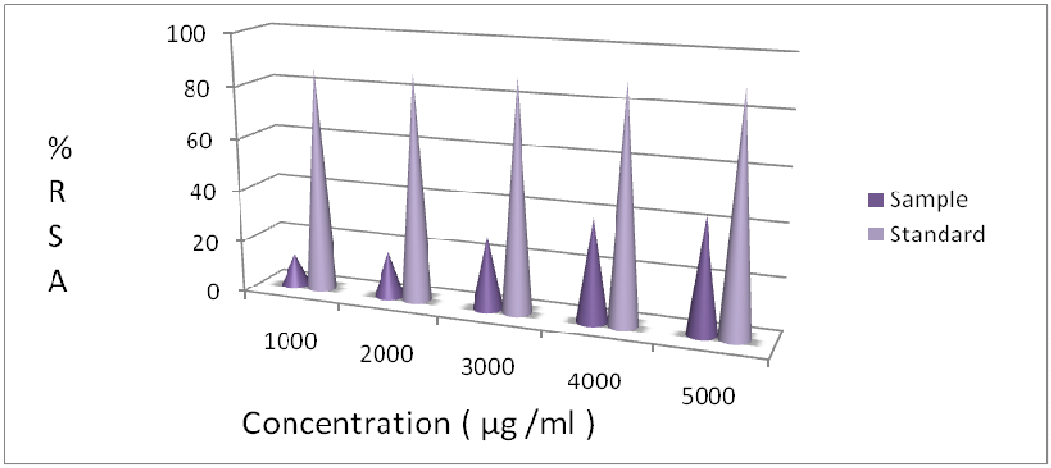


Figure 3
DPPH Radical Scavenging Activity of V. elaeagnifolia

4. GC-MS Analysis

On mass spectrum GC-MS was concluded using the data base of National institute standard Interpretation and technology (NIST).The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library .The 8 compound were identified in *V. elagenfolia* leaf extract by GCMS analysis are presented in Table no 4. The active principle with their retention time (RT), molecular formula, molecular weight (MW) and peak area or concentration (%) was taken into account. The major chemical constituents identified in leaves extract of V. elaeagnifolia were 14,15-Diepoxyregn-16-en-20-one,3,11a, 18triacetoxy(10.64%), Mibemycin, b,13-choro-5-demethoxy-28-epoxy-5(hydroxyimino)-25-(1-methylethy) (15.12%), 9-Desoxo-9-xi-hydroxy-3,7,8,9,12-Pentaacetate ingol (8.87%), 9-Desoxo-9-x-acetoxy3,8,12,tri-o-acetyingol,(11.19%)Propanoicacid,2-(3-acetoxyl-4,4,14-trimethyllandrost-8-en-17yl(13.41%),

Cyclopropal(3,4) benz(1,2,e)azulene-5,7b,9,9a-tetra, (acetyloxy)methyl)1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-9,9a-diacetate(16.55%), Phorbol12,13-dihexanolate(19.84%), 7,8-Epoxylanostan-11-01,3-acetoxy(4.38%).

Table 4
Chemical Composition of V. elaeagnifolia leaves

Sr. No	Retention Time	Name of chemical constituent	Molecular Formula	Molecular Weight	Peakarea %
1.	21.03	14,15-Diepoxyregn-16-en-20-one,3,11a,18 triacetoxy.	$C_{27}H_{34}O_9$	501.76	10.64
2.	23.3	Mibemycin, b,13-choro-5-demethoxy-28-epoxy-5(hydroxyimino)-25-(1-methylethy),(6R, 1R, 25R)	$C_{33}H_{46}ClNO_7$	602.71	15.12
3.	25.38	9-Desoxo-9-xi-hydroxy-3,7,8,9,12-Pentaacetate ingol.	$C_{30}H_{42}O_{11}$	578.08	8.87
4	27.27	9-Desoxo-9-x-acetoxy3,8,12, tri-o-acetyingol	$C_{39}H_{42}O_9$	534.62	11.19
5	29.03	Propanoic acid,2-(3-acetoxyl-4,4,14-trimethyllandrost-8-en-17yl.	$C_{27}H_{42}O_4$	429.38	13.41
6	30.82	1H-Cyclopropal(3,4)benz(1,2,e)azulene-5,7b,9,9a-tetra,3-(acetyloxy)methyl)1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-9,9a-diacetate,	$C_{24}H_{32}O_6$	415.36	16.55
7	33.07	Phorbol12,13-dihexanolate	$C_{32}H_{48}O_{11}$	560.72	19.84
8	33.88	7,8-Epoxylanostan-11-01,3-acetoxy.	$C_{32}H_{54}O_4$	502.77	4.38

The leaf extract of *V. elaeagnifolia* contain chemical composition showing interesting biological property, some author stated that, the compound Phorbol 12,13-dihexanolate having capacity to reactivated the HIV latency. He found that phorbol effectively activated HIV-1-gen expression in latently injected cell [33] and 3-acetoxy-7,8-epoxylanostan-1-ol are highly lithophilic and can easily permeate lipoidan layer of skin and they may contributed significantly tropical anti-inflamentary effect[34]. milbemycin oxime for veterinary use on DOGS and CATS against external and internal parasites: worms, lice, mites and it is also acts as agonist of the GABA (gammaaminobutyric acid) neurotransmitter in nerve cells and also binds to glutamate-gated chloride channels in nerve and muscle cells of invertebrates. Diepoxyregn-16-en-20-one, 3, 11a, 18 triacetoxy is a Glycosides compound which are active and complex substances containing carbon, hydrogen and oxygen. They have characteristic actions on contractile forces of cardiac muscle [35].

CONCLUSION

The present study is the first report on the chemical composition of the plant extract from leaves of *V. elaeagnifolia* growing in India. The presence of biologically active molecules as major components in leaf extract due to which it seems high importance for medic purposes. The results obtained in this work conclude that the extraction yield of the different extraction solvents used was correlated with the antimicrobial activity observed. Of the isolates tested, only 3(MEE and EAE) revealed susceptibly to the extracts, with DMSOE vealing the highest susceptibility. Antioxidant compounds such as phenolic and flavonoids are known for their antibacterial activities which might explain the highest susceptibility of the isolates tested to the methanolic extracts that presented the potent values of these compounds. Hence the plant contains potential antibacterial components that may be useful for evalution of pharmaceutical for the therapy of ailments and also plant extracts can be used for the treatment of infections caused by the strains of the test bacterial organisms.

Abbreviations

Aqueous extract [AQE], Methanolic extract [MEE], Chloroform extract [CLE], Ethyl acetate extract [EAE], Dimethyl sulphoxide extract [DMSOE], Antibiotic [AB], Standard deviation [S.D], 2,2 Diphenyl -1-Picryl hydrazyl [DPPH], Inhibition Concentration [IC_{50}], radical scavenging activity [RSA], National institute standard and technology [NIST], Flame ionization detector [FID].

REFERENCES

1. Cowan MM, Plant products as antimicrobial agents. Clin Microbiol Rev,12: 564-82,(1999).
2. Vongsombath C, Hugo J, de Boera, Pålsson K,. Keeping leeches at bay: Field evaluation of plant-derived extracts against terrestrial blood-sucking leeches (Haemadipsidae) in Lao PDR. Acta Tropica,119: 178– 182,(2011).
3. Ilondu EM, Phytochemical composition and efficacy of ethanolic leaf extracts of some *Vernonia* species against two phytopathogenic fungi. J Biopest, 6(2): 165-172,(2013).
4. Latha LY, Darah I, Jain K, and Sasidharan S, Effects of *Vernonia cinerea* Less methanol extract on growth and morphogenesis of *Candida albicans*. European Review for Medical and Pharmacological Sciences, 15(5): 543 - 549,(2011).
5. Ilondu EM., Okoegwale EE, Some medicinal plants used in the management of dermatophytic diseases in Nigeria. African Journal of Environmental Studies, 3(1&2): 146-151, (2002).
6. Bankole MO, Ayodele MS, and Adejumo OT, The antimicrobial effects of some *Asteraceae* commonly eaten as vegetables in Southwest Nigeria on some enteric pathogens. Compositae Newsletter, 40: 56-63,(2003).
7. Iwalokun BA, Efedede BU, Alabi-Sofunde JA, Oduala T, Magbagbeola OA and Akinwade AI, Hepatoprotective and antioxidant activities of *Vernonia amygdalina* on acetaminophen induce hepatic damage in mice. Journal of Medicinal Food, 9(4): 524-530,(2006).
8. Ilondu EM, Arimoro FO, Sodjie AP, The use of aqueous extracts of *Vernonia amygdaline* in the control of saperolegniasis. African Journal of Biotechnology, 8(24): 7130-7132,(2009).
9. Khandekar U, Ghongade R, Katolkar S, Screening on antioxidant activity, antimicrobial activity and phytoconstituents of Cyathocline lyrata leaf. International Journal of chemical and pharmaceutical sciences, 4(3): 64-68,(2013).
10. Elliott D.J, Zaoutis T.E, Troxel A.B, Loh A, Keren R, Empiric Antimicrobial Therapy for Pediatric Skin and Soft- Tissue Infections in the Era of Methicillin-Resistant Staphylococcus aureus. Pediatrics, 123(6): 959-66,(2009).
11. Duong M, Markwell S, Peter J, Barenkamp S, Randomized controlled trial of antibiotics in the management of community-acquired skin abscesses in the pediatric patient. Ann Emerg Med, 55(5): 401-7,(2010).
12. Paakkonen M, Kallio PE, Kallio MJ, Peltola H, Management of Osteoarticular Infections Caused by Staphylococcus aureus Is Similar to That of Other Etiologies: Analysis of 199 Staphylococcal Bone and Joint Infections. Pediatr Infect Dis J, 31(5): 436-8,(2012).
13. Kemper AR, Dolor RJ, Fowler VG, Jr. Management of skin abscesses by primary care pediatrician. Clin Pediatr (Phila), 50(6): 525-8, (2011).
14. James HE, Walsh JW, Wilson HD, Connor JD, Bean JR, Tibbs PA, Prospective randomized study of therapy in cerebrospinal fluid shunt infection. Neurosurgery, 7(5): 459-63,(1980).
15. Sohail MR, Uslan DZ, Khan AH, et al, Management and outcome of permanent pacemaker and implantable cardioverter-defibrillator infections. J Am Coll Cardiol, 49(18):1851-9,(2007).
16. Aldave AJ, Stein JD, Deramo VA, Shah GK, Fischer DH, Maguire JI, Treatment strategies for postoperative Propionibacterium acnes endophthalmitis. Ophthalmology, 106(12): 2395-2401,(1999).
17. Cunha BA, Acute acalculous Cholecystitis. Infect Dis Prac, 21: 70-1,(1997).
18. Camins BC, Marschall J, De Vader SR, Maker DE, Hoffman MW, Fraser VJ, The clinical impact of fluoroquinolone resistance in patients with E coli

bacteremia. J Hosp Med, 6(6): 344-9,(2011).
19. Glandt M, Adachi JA, Mathewson JJ, Jiang ZD, Dicesare D, Ashley D, Enteroaggregative Escherichia coli as a cause of traveler's diarrhea: clinical response to ciprofloxacin. Clin Infect Dis, 29(2): 335-8,(1999).
20. Crouch Brewer S, Wunderink RG, Jones CB, Leeper KV, Ventilator-associated pneumonia due to Pseudomonas aeruginosa. Chest, 109(4): 1019-29,(1996).
21. Gavin PJ, Suseno MT, Cook FV, Peterson LR, Thomson RB, Left-sided endocarditis caused by Pseudomonas aeruginosa: successful treatment with meropenem and tobramycin. Diagn Microbiol Infect Dis, 47(2): 427-30,(2003).
22. Hoban DJ, Zhanel GG, Clinical implications of macrolide resistance in community-acquired respiratory tract infections. Expert Rev Anti Infect Ther, 4(6): 973-80,(2006).
23. Nordmann P, Cuzon G, Naas T, The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis, 9(4): 228-36, (2009).
24. Einstein BI, Enterobacteriaceae. In: Mandell GL, Bennett, JE, Dolin E, (eds). Mandell Douglas, and Bennett's, Principles and Practice of Infectious Diseases, 5th ed, Vol 2, New York, NY: Churchill Livingstone, 2294-2310,(2000).
25. Linam WM, Gerber MA, Changing epidemiology and prevention of Salmonella infections. Pediatr Infect Dis J, 26(8): 747-8,(2007).
26. Harborne IB, Phytochemical methods: A guide to modern techniques of plant analysis. Edn 2, Chapman and Hall, New York, 88-185,(1973).
27. Sofowara A, Medicinal Plants and Traditional Medicine in Africa, 2nd Edn, Spectrum Books Ltd, 289-300, (1993).
28. Cavalieri SJ, Manual of antimicrobial susceptibility testing 1st Edn, American Society for Microbiology, 39-52,(2005)
29. Tsukatani T, Suenaga H, Shiga M, Noguchi K, Ishiyama M, Ezoe T, Matsumoto,K, Comparison of the WST-8 colorimetric method and the CLSI brothmicrodilution method for susceptibility testing against drug-resistant bacteria. J. Microbiol Methods, 90: 160–166,(2012).
30. Sochor J, Ryvolova M, Kizek R, Fully Automated Spectrometric Protocols for Determination of Antioxidant Activity: Advantages and Disadvantages. Molecules, 15: 8618-8640,(2010).
31. Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P, Antioxidant activity in vitro of the selenium-contained protein from the se-enriched bifidobacterium animals. Anaerobe,16 (4): 380-386,(2010).
32. Abirami P, Rajendra A, GC-MS analysis of methanol extract of vernonia cinera. European Journal Experiment biology, 2(1): 9-12, (2002).
33. Márquez N, Calzado MA, Sánchez-Duffhues G, Pérez M, Minassi A, Pagani A, Appendino G, Diaz L, Munoz-Fernandez M.A, Muñoz E, Differential effects of phorbol-13-monoesters on human immunodeficiency virus reactivation. Biochem Pharmacol, 15; 75(6): 1370-80,(2008).
34. Osadebe PO, Okoye FBC, Topical anti-inflammatory effect of lipophilic constituents from Alchornea floribunda and Alchornea cordifolia leaves. J. Ethnopharmacol, 89: 19–24,(2003).
35. Hikino H, Kiso Y, Natural products for liver diseases in Economics and Medicinal Plants Research. Vol. 2nd. In: H Wagner, H Hikino and NR Famsworth (Eds.). Wagner, Academic Press: 39-72, (1988).