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PHYTOCHEMICAL ANALYSIS AND ANTITUBERCULAR ACTIVITY OF *CENTRATHERUM ANTHELMINTICM* SEED EXTRACT

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ABSTRACT: About 4 extracts (hexane, acetone, ethanol, and methanol extraction) from the seeds of *Centratherum anthelminticum* was extracted. Gas Chromatography-Mass Spectroscopy characterized ethanol extract. Seven constituents from 9 peaks were identified. 2-Oxo-pentanoic acid and hexadecanoic acid are as the major constituents, and the minor constituents like Henicosanoic acid, 26-Phenoxy-hexacosanoic methanoate, Hexadecanoic acid methyl ester, dotriacontanoic acid ethyl ester, nonadec-3-ene, icosa-2,5, diene, and tetratriacontane were identified. The antimicrobial activity of the different extract was tested against human and plant pathogenic bacteria. Ethanol extract showed a significant role in inhibiting almost all tested pathogenic organisms and antitubercular activities at various concentration.

INTRODUCTION: *Centratherum anthelminticum (*L.) Kuntze is an ethnomedicinal plant commonly grown in India and Southeast Asia. It belongs to Asteraceae family, and its seeds are known as Kalijiri in Hindi [1, 2], *Vernonia anthelmintica*, and *Conyza anthelminticum* are scientific synonyms of this plant. The plant is an erect, pubescent, annual herb which can grow up to 90 cm in height and the seed are brownish, with a hot, sharp taste and astringent properties. The major chemical constituents present in this plant are glycosides, carbohydrates [3], phenolic compounds, and tannins, flavonoids [4], proteins, saponins, sterols [5], lipids, fats [6], sesquiterpene lactones, alkaloids [7], terpenoids [8] and steroids [9].

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The seeds of *C. anthelminticum* have been reported for many pharmacological activities like anti-inflammatory activity [10], anti-arthritic activity [11], anti-pyretic [12], anti-filarial [13], anti-cancer [14], anti-microbial [15], anti-malarial [16], anti-viral [17], anthelmintic [18], anti-diabetic [19], melanogenesis [20], wound healing activity [21], anti-bacterial and anti-fungal activities [22, 29], anti-hypoglycemic activity [23], diuretic agent [24], analgesic [25], larvicidal activity [26], cytotoxic activity [27], and anti-implantation activity [28].

MATERIALS AND METHODS:

Plant Material: The dried seeds of *C. anthelminticum* were collected from the local market of Ujjain **Fig. 1, 2**.

Seeds Extract: An amount of 500g of fresh seeds was weighed and shade dried, cleaned, and powdered coarsely. The powdered seeds were extracted by hexane, acetone, ethanol and methanol extraction for 95-126 h each in a Soxhlet extractor (40-60 ºC). The solvent was removed by rotary film

evaporator, and concentrated extracts were preserved in refrigerator for further use.

FIG. 1: *C. ANTHELMINTICUM* PLANT

FIG. 2: *C. ANTHELMINTICUM* SEEDS

Analysis of Ethanol Extract: Mass spectrometry analysis was performed on Shimadzu GCMS-QP-2010 SE model using Direct Injection Probe Technique.

Antimicrobial Activity: The different seed extracts were subjected to the antimicrobial assay followed by agar well diffusion method [30]. 38 gm of Muller Hinton Agar was suspended in 1000ml of distilled water and heated up to boiling point for complete mixing. To sterilize, it was autoclaved at 15 lbs pressure at 121 ºC for 15 min. 100 mg of each extract was suspended in 5ml of 10% DMSO. Approximately 25 ml of sterilized selective medium was poured into each Petridis and solidified at room temperature. Using a sterile cotton swab, the bacterial culture was swabbed on the surface of pre-poured nutrient agar plates. The plates were allowed to dry for 15 min, before use in the test. A well of 10mm diameter punched off at previously marked Petri plates into agar medium with the sterile cup before then it was filled with 100 ul of extract every time. Plates were places for 30 min in the refrigerator for diffusion of extracts and then incubated at 37 ºC for 24 h. Zone of inhibition (excluding well diameter) formed was measured as a property of the antibacterial and antifungal activity.

Antitubercular Activity: The different seed extracts of *C. anthelminticum* were screened for antitubercular activity against *Mycobacterium tuberculosis* $H_{37}R_V$ strain using Lowenstein–Jensen medium method [31]. Ten mg of each extract was dissolved in 10 ml of DMSO to get a concentration of 1000 ug/l. Further dilutions were made with DMSO to get different concentrations such as 100, 10, and 1 ug/ml. 0.8 ml of each concentration was used for the study. To this, 7.2 ml of Lowenstein–Jensen medium was added.

RESULT AND DISCUSSION: In the present study, an amount of 500g of *C. anthelminticum* seeds and solvents such as hexane, acetone, ethanol, and methanol were used for the extraction. From each sample 10 ml, extracts were collected for screening biological activities.

GC-MS analysis of Ethanol Extract: GC-MS analysis indicated that the ethanol extract contained about 9 peaks. The composition of ethanol extract and its retention time are given in **Table 1**.

TABLE 1: COMPOSITION OF *C. ANTHELMINTICUM* ETHANOL EXTRACT

Number of Peaks	Retention Time (minutes)	Compounds
1	4.405	2-Oxo-pentanoic acid
2	5.145	Henicosanoic acid
3	6.430	26-Phenoxy-hexacosanoic methanoate
4	15.230	Hexadecanoic acid methyl ester
5	15.635	hexadecanoic acid
6	16.060	dotriacontanoic acid ethyl ester
7	17.400	nonadec-3-ene
8	18.940	icosa-2,5,diene
9	20.470	tetratriacontane

2-Oxo-pentanoic acid, Henicosanoic acid, 26-Phenoxy-hexacosanic methanoate, Hexadecanoic acid methyl ester, hexadecanoic acid, dotriacontanoic acid ethyl ester, nonadec-3-ene, icosa-2, 5, diene, and tetratriacontane were identified.

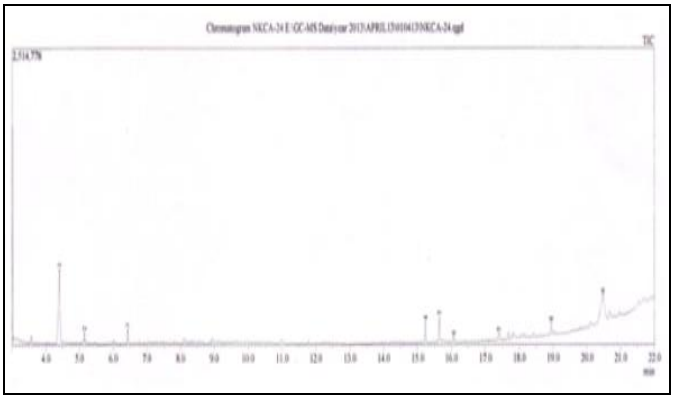

FIG. 3: CHROMATOGRAM OF *C. ANTHELMINTICUM* (SEEDS) ETHANOL EXTRACT

Antimicrobial Activity of Seed Extracts of *C. anthelminticum*: In the present study, the antimicrobial activities of different extracts of *C. anthelminticum* were tested against nine bacteria (*Staphylococcus albus, Staphylococcus aureus, Staphylococcus heamolyticus, Vibrio cholera, Pseudomonas aerusinosa, Klebisella aerogenes, Escherichia coli, Pseudomonas pyocyneaus, Diplococcus peunoniae*). It was clear from the present result that ethanol extract exhibited pronounced activity against all the bacteria. The presence of phytoconstituents in the seed extracts may be responsible for the antibacterial activity of the plant. It has been documented that different solvents have diverse solubility capacities for different phytoconstituents. The result was represented in **Table 2.**

TABLE 2: ANTIMICROBIAL ACTIVITY OF *C. ANTHELMINTICUM* SEEDS EXTRACTS AGAINST 9 BACTERIAL STRAIN BY AGAR WELL DIFFUSION METHOD

Microorganisms	Zone of inhibition in 100µl of 20mg/ml(mm)			
	Hexane extract	Acetone extract	Ethanol extract	Methanol extract
Staphylococcus albus	13.0	3.0	14.0	3.0
Staphylococcus aureus	3.0	14.0	16.0	11.0
Staphylococcus heamolyticus	11.0	14.0	9.0	14.0
Vibrio cholerae	2.0	3.0	6.0	3.0
Pseudomonas aerusinosa	3.0	3.0	8.0	5.0
Klebisella aerogenes	8.0	4.0	4.0	5.0
Escherichia coli	6.0	6.0	8.0	12.0
Pseudomonas pyocyneaus	5.0	6.0	3.0	3.0
Diplococcus peunoniae	3.0	5.0	9.0	5.0

Antitubercular Activity: Pyrazinamide was used as the standard drug. The dilution of Pyrazinamide was made with DMSO to get different concentrations of 100, 10, and 1 µg/ml. 0.8 ml of each concentration was used for the study.

A sweep from the *Mycobacterium tuberculosis* $H_{37}R_V$ culture was discharged with the help of nichrome wire loop with a 3 mm external diameter, into a sterile distilled bijou bottle containing 6 mm glass beads and 4 ml of sterile distilled water. The bottle was shaken with the help of a mechanical shaker for 2 min, and then using nichrome wire loop, 3 mm external diameter, a loopful of the suspension was inoculated on the surface of each of Lowenstein–Jensen medium containing the test compounds **Fig. 4**. Lowenstein–Jensen medium Containing pyrazinamide as well as control were inoculated with *Mycobacterium tuberculosis* $H_{37}R_V$ strain. The inoculated medium was incubated at 37ºC for 4 weeks. At the end of 4 weeks, readings were taken and recorded in **Table 3**.

TABLE 3: ANTITUBERCULAR ACTIVITY OF DIFFERENT SEEDS EXTRACT OF *C. ANTHELMINTICUM*

Compound	*Mycobacterium tuberculosis* concentration in µg/mL		
	100	10	1
Control	+++	+++	+++
Hexane extract	-ve	-ve	-ve
Acetone extract	-ve	-ve	-ve
Ethanol extract	-ve	-ve	-ve
Methanol extract	-ve	-ve	-ve

+++ indicates intensive growth of *M. tuberculosis*
-ve indicates complete inhibition of $H_{37}R_V$

FIG. 4: ANTITUBERCULAR ACTIVITY

CONCLUSION: From this study, it can be concluded that the ethanol extract of *Centratherum anthelminticum* seeds exhibited pronounced activity against all the tested bacteria.

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CONFLICT OF INTEREST: Nil

REFERENCES:

1. The Wealth of India – Raw Materials, First Supplement Series, National Institute of Science Communication and Information Resources, CSIR, New Delhi 2004; 5: 294.
2. Chopra RN, Nayar SL and Chopra IC: Glossary of Indian Medicinal Plants, CSIR Publication, New Delhi 2002: 58-59.
3. Yadava RN and Barsainya D: Analysis of carbohydrates from seeds of *Centratherum anthelminticum* Kuntze. Asian J Chem 1996; 8(4): 813-814.
4. Tian G, Zhang U, Zhang T, Yang F and Ito Y: Separation of flavonoids from seeds of *Vernonia anthelmintica* Willed. By high-speed counter-current chromatography, J. Chromatogr A 2004; 1049(1-2): 219-222.
5. Akihisa T, Hayashi Y, patterson GW, Shimizo N and Tamura T: sterols from *Vernonia anthelmintica* seeds. Phytochemistry 1992; 31(5): 1759-1763.
6. Sing C and Kaul BL: Oil and seed meal composition of *Centratherum anthelminticum*. J Med Arom Plant Sci 1999; 21: 308-310.
7. Johry RK and Singh C: Medicinal uses of vernonia species. J Med Arom Plant Sci 1997; 19: 744-752
8. Mehta BK, Mehta D and Itoriya A: Structure elucidation by NMR spectroscopy of a new acetylated saponin from *Centratherum anthelminticum*. Carbohydrate Research 2004; 339(18): 2871-2874.
9. Mehta BK, Mehta D and Verma M: Novel steroids from the seeds of *Centratherum anthelminticum*. Natural Product Research 2005; 19(5): 435-442.
10. Ashok P, Koti BC, Thippeswamy AH, Tikare VP, Dabadi P and Vishvanathaswamy AH: Evaluation of anti-inflammatory activity of *Centratherum anthelminticum* L. Kuntze seeds. Indian J Pharm Sci 2010; 72: 697-703.
11. Otari KV, Shete RV, Upasani CD, Adak VS, Bagade MY and Harpalani AN: Evaluation of anti-inflammatory and anti-arthritic activities of ethanolic extract of *Vernonia anthelmintica* seeds. J. Cell Tissue Res 2010; 10(2): 2269-228.
12. Purnima A, Koti BC, Tikare VP, Viswanathaswamy AH, Thippeswamy AH and Dabadi P: Evaluation of analgesic and antipyretic activities of *Centratherum anthelminticum* (L.) Kuntze seed. Indian J Pharm Sci 2009; 71: 461-464.
13. Mehta BK, Mehta D and Itoriya A: isolation structure determination of acetylated triterpenoid saponin from the seeds of *Centratherum anthelminticum*. Nat Prod Res 2010; 24: 120-130
14. Looi CY, Arya A, Cheah FK, Muharram B and Leong KH: Induction of apoptosis in human breast cancer cells *via* caspase pathway by vernodalin isolated from *C. anthelminticum* L. seeds. Plos One 2013; 8(2): 56643
15. Ani V: Studies on phytochemicals and biological properties of bitter cumin *Centratherum anthelminticum* L. Kuntze Ph.D. thesis, University of Mysore, India 2008.
16. Clement E, Erharuyi O, Vincent I, Joy A, Christopher A, Anthony A, Onyekaba, Osakue J, Iftikhar A and Abiodun F: Significance of bitter leaf (*Vernonia amagdalina*) in tropical diseases and beyond: a review. Malar Chemoth Cont 2014; 3(1): 2-10.
17. Bhakuni DS, Dhar ML, Dhar MM, Dhavwan BN and Mehrotra BN: Scrrening of Indian plants for biological activity part II. Ind J Bio1969; 7: 250-262
18. Iqbal Z, Lateef M, Jabbar A, Akhtar MS and Khan MN: Anthelmintic activity of *Vernonia anthelmintica* seeds against trichostrongylid nematodes of sheep. Pharma Biol 2006; 44: 563-567.
19. Arya A, Looi CY, Wong WF, Noordin MI, Nyamathulla S, Mustafa MR and Mohd MA: *In-vitro* anti oxidant, PTP-1B inhibitory effects and *in-vivo* hypoglycemic potential of selected medicinal plants. Int J Pharmacol 2013; 9: 50-57.
20. Zhou J, Shang J, Ping F and Zhao G: Alcohol extract from *Vernonia anthelmintica* (L.) willd seed enhances melanin synthesis through activation of the p38 MAPK signaling pathway in B16F10 cells and primary melanocytes. J Ethanopharmacol 2012; 143: 639-647.
21. Sahoo HB, Sagar R and Patel VK: Wound healing activity of *C. anthelminticum*, Linn. Mol Clin Pharm 2012; 3: 1-7.
22. Singh O, Ali M and Husain SS: Phytochemical investigation and antifungal activity of the seeds of *Centratherum anthelminticum* Kuntze. Acta Poloniae Pharmaceutica 2012; 69: 1183-1187.
23. Akram MH, Shamim AQ: *Centratherum anthelminticum* minimizes the risk of insulin resistance in fructose-induced type 2 diabetes. J Applied Pharm Sci 2015; 5: 74-78.
24. Koti BC and Purnima A: Diuretic activity of an extract of *Centratherum anthelminticum*. Int J Green Pharmacy 2008; 2: 228-231.
25. Pitchai G, Paydar M, Moharam BA, Wong YL, Looi CY, Wong WF, Nyamathulla S, Pandy V, Pitchai D, Manikkam R and Rajendran SR: Database on pharmacophore analysis of active principles, from medicinal plants. Bioinformation 2010; 5: 43-45.
26. Anamika S, Roli B, Shobhita T, Srivastava SS and Kumar KM: Larvicidal activity of an indigenous plant, *C. anthelminticum*. J of Envi. Biology 2008; 29(5): 669-672.
27. Chung YL, Bushra M, Mahammad JP, Yi LW, Kok HL, Khalit M, Aditya A, Won FW and Mohd RM: Induction of apoptosis in melanoma A375 cells by a chloroform fraction of *Centratherum anthelminticum* L. seeds involves NF-kappaB, p53 and Bcl-2-controlled mitochondrial signaling pathways. BioMed Central 2013.
28. Mehta BK, Sharam S and Gupta DN: Screening of post-coital anti-implantation activity of *Machela champaka* and *Centratherum anthelminticum* (seeds). Indian Drugs 1994; 31: 280-281.
29. Mehta BK and Sharam S: In-vitro antimicrobial efficacy of *Centratherum anthelminticum* (seeds) extracts, Journal of Hygiene, Epidemiology, Microbiology, and Immunology 1991; 35(2): 157-161.
30. Perez C, Pauli M and Bazerque P: An antibiotic assay by the agar-well diffusion method. Acta Biologiae et Medecine Experimentaalis 1990; 15: 113-115.
31. Cambau E, Truffot-Pernot C, Boulahbal F, Wichlacz C, Grosset J and Jarlier V: Mycobacterial growth indicator tube versus the proportion method on Lowenstein-Jensen medium for antibiotic susceptibility testing *Mycobacterium tuberculosis*. Eur J Clin Micro Inf Dis 2000; 12: 938–942.

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