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In vitro antimicrobial and anthelmintic activity of steam distillates of *Hemidesmus indicus* and *Swertia chirata* alone and in combination with Cow urine

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ABSTRACT

Essential oils are valuable natural products used as raw materials in many fields. This study reveals antimicrobial and anthelmintic activity of steam distillates of *Hemidesmus indicus* and *Swertia chirata* alone and in combination with Cow urine. The steam distillates were obtained by subjecting the powders of plants to distillation using simple distillation apparatus. The distillates obtained were tested for antibacterial activity, antifungal activity and anthelmintic activity. The results obtained revealed potential of the distillates against bacteria, fungi and worm tested. Significant result was seen in case of *Hemidesmus* distillate when compared to other distillates. *Hemidesmus* distillate alone was found to be superior to distillate from *Hemidesmus*-Cow urine combination. *Swertia* distillate was found to possess lesser activity when compared to combination. Among fungi tested, *Aspergillus niger* was found to be affected to more extent than other fungi. The results revealed the potential of steam distillates against bacteria, fungi and helminthes. The inhibitory effect of distillates could be attributed to active principles present.

KEYWORDS

Hemidesmus indicus;
Swertia chirata;
Antimicrobial activity;
Anthelmintic activity;
Steam distillates.

INTRODUCTION

Hemidesmus indicus commonly known as Indian Sarsaparilla, belonging to the family Asclepiadaceae, is a slender laticiferous, twining, sometimes prostrate or semi erect shrub, occurring over the greater part of India. This is a common medicinal plant widely used in Indian Systems of Medicine and also an official drug in Indian Pharmacopoeia and British Pharmacopoeia. The roots are used as antipyretic, anti-diarrhoeal, astringent, blood purifier, diaphoretic, diuretic, refrigerant and tonic. Roots are useful in biliousness, blood diseases, dysentery, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, leprosy, leucoderma, leucorrhoea, itching, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism. Root bark is used to cure dyspepsia, loss of appetite, nutritional disorders, fever, skin diseases, ulcer, syphilis, rheumatism and leucorrhoea. Stem of *H.indicus* is used as dia-

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phoretic, diuretic, laxative and in treating brain, liver and kidney diseases, syphilis, gleet, urinary discharges, uterine complaints, leucoderma, cough and asthma[1]. *Swertia chirayita* belongs to family Gentianaceae, which records the occurrence of taxonomically informative molecules, namely iridoids, xanthones, mangiferin and C-glucoflavones. The biological activities attributed to *Swertia chirayita* are Alternative, Antihelmintic, Antileishmanial, Anticholinergic, Anticonvulsant, Antiedemic, Antiinflammatory, Antimalarial, Antipyretic, Antitubercular, Astringent, Bitter, Cardio stimulant, Cholagogue, Choleretic, CNS depressant, Emollient, Hepatoprotective, Hypnotic, Hypoglycemic/antidiabetic, Laxative, Secretagogue, Stomachic, Tonic, Undersedative, Vermifuge. *S.chirayita* is used in British and American pharmacopoeias as tinctures and infusions. According to Ayurvedic pharmacology, chirata is described as bitter in taste (*rasa*). The thermal action (*virya*) of chirata is defined as cooling (*shita*). Chirata is light (*laghu*), i.e. easily digestible, and *ruksha* (*dry*). These characteristics drain heat from the blood and liver. Its use has also been mentioned in Unani medicine. Concoction of chirata with cardamom, turmeric and kutki is given for gastrointestinal infections, and along with ginger it is considered good for fever. When given along with neem, manjishta and gotu kola, it serves as a cure for various skin problems. It is used in combination with other drugs in cases of scorpion bite[2].

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition[3]. Essential oils are products, generally, of rather complex composition comprising the volatile principles contained in the plants, and more or less modified during the preparation process[4].

The aims and objectives of present study carried by us is prepare steam distillates of *Hemidesmus indicus* and *Swertia chirata* alone and in combination with Cow urine and find out whether the possess antimicrobial and anthelmintic activity.

MATERIALS AND METHODS

Extraction by steam distillation

Essential oils can be extracted using a variety of methods, although some are not commonly used today. Currently, the most popular method for extraction is steam distillation in which water is heated to produce steam that carries the most volatile chemicals of the aromatic material with it. The steam is then chilled (in a condenser) and the resulting distillate is collected. The Essential Oil normally float on top of the Hydrosol (the distilled water component) and may be separated off[5]. A simple laboratory quick-fit apparatus (Figure 1) with a 1000ml distilling flask (to boil the mixture of plant material and water), a condenser (to condense the steam to obtain the steam distillate), and a receiving vessel, was used for the steam distillation. A known weight of (100 grams) air-dried plant material was subjected to steam distillation in the assembly. When heated up, the plant cells release their components and some of them are volatilized and carried by the steam. The volatile components were collected into the receiving flask during 3 hours of steam distillation[6,7]. Steam distillation was carried using powders of the selected plants and combination of powder and Cow urine. The distillates were transferred into clean containers and stored in refrigerator until use. In case of some plant materials, the recovered mixture was allowed to settle and the oil was withdrawn. The recovered essential oils were also maintained in the refrigerator for future use.

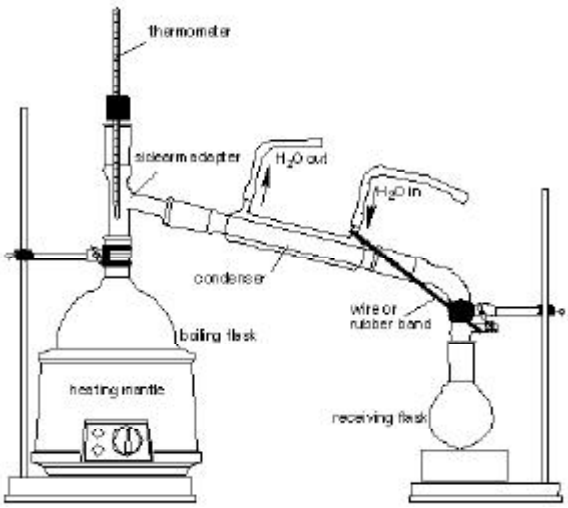

Figure 1: Distillation assembly

Screening for antibacterial activity

Gram positive bacteria namely *Bacillus subtilis, Staphylococcus aureus* and Gram negative bacteria namely *Escherichia coli, Klebsiella pneumoniae and*

Pseudomonas aeruginosa were used. The pure cultures of test bacteria on Nutrient agar slants were maintained in refrigerator and regularly checked for contamination. Periodic transfers were made aseptically. Test tubes containing sterile Nutrient broth were aseptically inoculated with the pure cultures of test bacteria maintained on nutrient agar slants and incubated at 37^0C for 24 hours. The broth cultures of test bacteria obtained after incubation were used for inoculation. The antibacterial activity of steam distillates was tested in liquid nutrient media[8] with minor modifications. The nutrient broth containing known volume of steam distillate of selected plants and plant-cow urine combination was sterilized by autoclaving. The sterile media containing tubes were inoculated with standardized volumes of 24 hours old broth cultures of test bacteria followed by incubation at 37^0C for 24 hours. A set of nutrient broth tubes inoculated with bacterial cultures was kept as control without adding steam distillates. After incubation, the contents in the tubes were mixed thoroughly using vortex mixer and the optical density was measured by spectrophotometer at a wavelength of 490 nm as a guide to microbial growth. The whole set of experiments was performed in triplicate, taking the means to get reliable results, and each set included a control broth containing no steam distillate.

Screening for antifungal activity

In the study, species of the genus *Aspergillus* were selected as target fungi which are known to cause opportunistic mycotic infections in susceptible individuals. The pure cultures of test fungi on SDA slants and were maintained in refrigerator. Periodic subcultures were done aseptically. The suspension of spores of the test fungi (for inoculation on poisoned plates) was prepared in a test tube containing 0.85% sterile normal saline containing 0.01% Tween 80 detergent[9]. The antifungal activity was assessed using Poison food technique[10]. The test fungi was allowed to grow in Sabouraud's dextrose agar plates poisoned with steam distillates (10% concentration). The test fungi were inoculated by Point inoculation method where the spore suspension of test fungi were taken with the help of a sterile inoculation needle and touched the centre of the medium. The effect of extract on fungal growth was determined by measuring the diameter of the colony obtained on poisoned plate and comparing with control (plates not poisoned with extract). The experiment was done in triplicate and average reading was recorded.

Screening for anthelmintic activity

In this study, Indian earthworm model was selected as the earthworms are easily available and used widely for the initial evaluation of anthelmintic activity of compounds. The assay was performed on adult Indian earthworm due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings[11]. Indian adult earthworms (*Pheretima pasthuma*) collected from the local earthworm breeder in the outskirts of Shivamogga city were used for the Anthelmintic study. Equal sized (8± 1 cm) worms were selected for the study. The worms were washed with normal saline to remove all the extraneous matter. Piperazine citrate (750mg/5ml) manufactured by GlaxoSmithKline Pharmaceutical Limited, Bangalore, was used as reference standard for anthelmintic study. 0.85% normal saline was used as control. It was prepared by dissolving 0.85 g NaCl in minimum volume of distilled water and the final volume was made up to 100ml with distilled water.

Various dilutions of standard drug (Piperazine) and test materials (steam distillates) were prepared in normal saline (0.85%). 5% of standard drug and test in normal saline were poured into respective labeled Petri plates (50 ml in each plate) and 6 worms of equal size (or nearly equal) were introduced into each of the plates. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur *when the worms were not able to move even in normal saline*. Death was concluded *when the worms lost their motility followed with fading away of their body colors*[11]. Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased[12].

RESULTS AND DISCUSSION

Results of antibacterial activity of steam distillates of selected plants alone and in combination with cow urine against Gram positive and Gram negative bacteria is depicted in TABLE 1. Among distillates employed,

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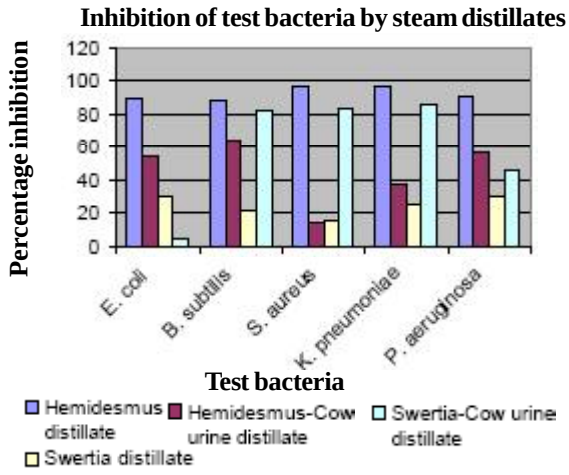
TABLE 1: Antibacterial activity of steam distillates of selected plants (single and combined distillates)

Treatment*	Optical density at 540nm				
	E coli	*B.subtilis*	*S.aureus*	*K.pneumoniae*	*P.aeruginosa*
Control	0.440	0.488	0.625	0.585	0.505
Hemidesmus distillate	0.047 (89.31)	0.060 (87.70)	0.020 (96.80)	0.020 (96.58)	0.046 (90.89)
Hemidesmus-Cow urine distillate	0.200 (54.54)	0.173 (64.54)	0.535 (14.40)	0.365 (37.60)	0.217 (57.03)
Swertia distillate	0.305 (30.68)	0.380 (22.13)	0.530 (15.20)	0.435 (25.64)	0.350 (30.69)
Swertia-Cow urine distillate	0.108 (75.45)	0.090 (81.55)	0.103 (83.52)	0.079 (86.48)	0.270 (46.53)

Results are average of three trials, Values within parentheses- Percentage inhibition when compared to control, *Concentration- 5%

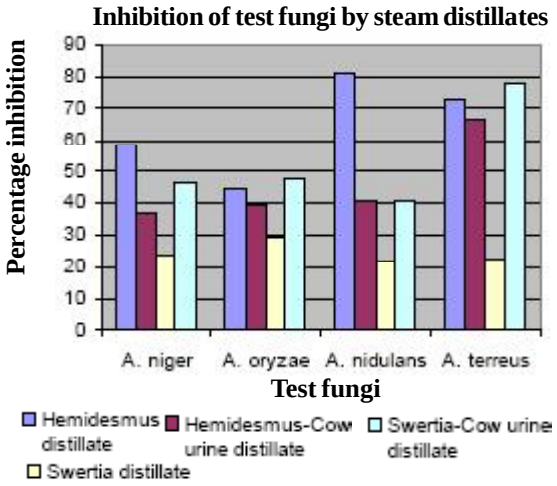


more inhibition was recorded in case of *Hemidesmus indicus* when compared to *Swertia chirata*. An inhibition of more than 80% was recorded in case of all bacteria by *Hemidesmus* steam distillate. But distillate of *Hemidesmus*-Cow urine combination was not found to be effective as the inhibition recorded was lesser when compared to *Hemidesmus* distillate alone. In case of *Swertia*, combination trial was found to be more effective than *Swertia* distillate alone. This suggests that the *Hemidesmus* distillate alone is more potent than combination while combination trial was effective than plant distillate alone in case of *Swertia*. In case of antifungal activity (TABLE 2), the colony diameter of test fungi was found to be less in poisoned plates when compared to control. *A.niger* was found to be more inhib-

TABLE 2: Antifungal activity of steam distillates of selected plants (single and combined distillates)

Treatment*	Average colony diameter in cm			
	A.niger	*A.oryzae*	*A.nidulans*	*A.terreus*
Control	4.3	3.8	3.7	2.7
Hemidesmus distillate	1.8 (58.14)	2.1 (44.73)	0.7 (81.08)	0.7 (73.07)
Hemidesmus-Cow urine distillate	2.7 (37.20)	2.3 (39.47)	2.2 (40.50)	0.9 (66.66)
Swertia distillate	3.3 (23.25)	2.7 (28.94)	2.9 (21.62)	2.1 (22.22)
Swertia-Cow urine distillate	2.3 (46.51)	2.0 (47.36)	2.2 (40.50)	0.6 (77.77)

Results are average of three trials, Values within parentheses- Percentage inhibition when compared to control, *Concentration- 10%

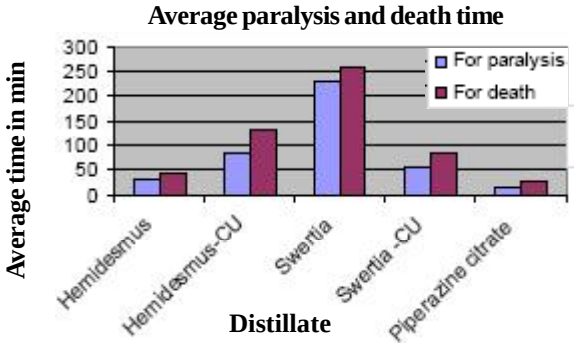


ited by distillates when compared to other test fungi. More inhibition of test fungi was recorded in case of *Hemidesmus* steam distillate when compared to other trials. *Swertia* distillate alone was found to be less inhibitory as compared to *Hemidesmus* distillate. In combination trials, *Swertia* was found to be superior to *Hemidesmus*. The results of the study reveal that *Hemidesmus* distillate alone was more effective than combination trial. TABLE 3 reveals anthelmintic activity of steam distillates against earthworm model. The average paralysis and death time in case of *Hemidesmus* distillate alone was found to be 31 and 43 minutes respectively while it took more time to cause paralysis (85 min) and death (133 min) in case of *Hemidesmus*-Cow urine combination. In this case also *Hemidesmus* distillate alone was found to be more effective than combination trial. Paralysis and death time was found to be less in case distillate from Swertia-Cow urine combination than *Swertia* distillate alone. Thus, combination trial was found to be effective only

TABLE 3 : Anthelmintic activity of various extracts of selected plants (single and in combination)

Treatment*	Average time (in minutes)	
	For paralysis	For death
Hemidesmus Distillate	31	43
Hemidesmus-Cow urine distillate	85	133
Swertia Distillate	229	257
Swertia -Cow urine distillate	56	83
Piperazine citrate	16	28

Results are average of three trials, Number of worms in each trial- 6 worms/treatment, *Concentration- 5%



in case of *Swertia* while *Hemidesmus* distillate alone was more effective than combination.

Herbal distillates are aqueous solutions or colloidal suspensions (hydrosol) of essential oils usually obtained by steam distillation from aromatic plants. Aromatherapy is the therapeutic use of fragrances or at least mere volatiles to cure, mitigate or prevent diseases, infections and indispositions by means of inhalation[13]. This has recently attracted the attention of many scientists and encouraged them to screen plants to study the biological activities of their oils from chemical and pharmacological investigations to therapeutic aspects. Essential oils are complex mixtures comprising many single compounds. Each of these constituents contributes to the beneficial or adverse effects of these oils. Therefore, the intimate knowledge of essential oil composition allows for a better and specially directed application[3]. They are essentially obtained by hydrodistillation (the plant material is heated in two to three times its weight of water with indirect steam from outside the still) as opposed to steam distillation (the plant material is extracted by direct steam, produced in the still, or by indirect steam, produced outside and fed into the still), hydrodiffusion (low-pressure steam (< 0.1 bar) replaces the volatile from the intact plant material by osmotic action) or CO_2 extraction[3]; in addition to expression of the pericarp (or cold pressing) which is a special method for *Citrus* (Rutaceae) peel oils extraction[14,15] from fresh or dried material. The microwave assisted process has also been developed and reported by many authors as a technique for extraction of essential oils in order to obtain a good yield of the essence and to reduce time of extraction[16,17]. Another technique consists of extracting oils using a mechanical and thermochemical reaction[17]. Chemical analysis of essential oils is generally performed using GC for quantitative analysis and GC/MS for qualitative analysis[18].

CONCLUSION

Essential oils are complex mixtures comprising many single compounds. Each of these constituents contributes to the beneficial or adverse effects of these oils. This has recently attracted the attention of many scientists and encouraged them to screen plants to study the biological activities of their oils from chemical and pharmacological investigations to therapeutic aspects. The present investigation reveals the potential of these herbal distillates against bacteria, fungi and worms tested. Further experiments in animal models could possibly justify the real potential of the plants selected in inhibiting disease causing pathogens.

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REFERENCES

[1] A.Austin; J.Biol.Sci., **8(1)**, 1-12 **(2008)**.
[2] P.Joshi and V.Dhavan; Current Science, **89(4)**, 635-640 **(2005)**.
[3] G.Buchbaur; Perfumer and Flavorist, **25**, 64-67 **(2000)**.
[4] J.Bruneton; Pharmacognosy, Phytochemistry, Medicinal Plants.Intercept, Ltd., Hampshire, **(1995)**.
[5] K.F Abed; Saudi Journal of Biological Sciences, **14 (1)**, 53-60 **(2007)**.
[6] P.Zeinsteger, A.Romero, P.Teibler, M.Montenegro, E.Rios, E.M.Ciotti, O.Acosta de Parez, N.Jorge;

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RIA., **32 (2)**, 125-136 **(2003)**.

[7] H.Peng and X.Yang; J Zhejiang Univ Sci., **6B(2)**, 91-95 **(2005)**.

[8] C.M.M Ludin, J.M Radzi; Mal.J.Med.Sci., **8(2)**, 14-18 **(2001)**.

[9] Z.Rihakova, V.Filip, M.Plockova, J.Smidrkal; Czech.J.Food Sci., **20(2)**, 48-52 **(2002)**.

[10] G.Singh, S.Maurya, M.P DeLampasona, C.Catalan; J.Food Sci., **70(4)**, 208-215 **(2005)**.

[11] A.S.Girme, R.D.Bhalke, P.B.Ghogare, V.D.Tambe, R.S.Jadhav, S.A.Nirmal; Dhaka Univ.J.Pharm.Sci., **5(1-2)**, 5-7 **(2006)**.

[12] Temjenmongla., A.K.Yadav; Afr.J.Trad.Cam., **2(2)**, 129-133 **(2005)**.

[13] G.Buchbauer, W.Jager, L.Jirovetz, J.Imberger, H. Dietrich, R.Teranishi, R.Buttery, H.Sugisawa; 'Bioactive Volatile Compounds from Plants', 161 **(1993)**.

[14] A.Baaliouamer, B.Y, Meklati, D.Fraisse, C.Scharff; J.Essent Oil Res., **4**, 251-258 **(1992)**.

[15] E.Dellacassa, C.Rossini, P.Menendez; J.Essent Oil Res., **4**, 265-272 **(1992)**.

[16] J.R.Pare, M.Sigouin, J.Lapointe; Can.No.600322, **(1989)**.

[17] N.Bouzid, G.Vilarem, A.Gaset, B.Benjilali, M. Ettalibi, M.Ismaili-Alaoui, S.Zrira; 'Proceedings of the Intern.Congr.Arom.Medicinal Plants and Essential Oils', Actes Editions, Rabat, Morocco, 115-120 **(1997)**.

[18] G.Keravis, B.Benjilali, M.Ettalibi, M.Ismaili-Alaoui, S.Zrira; 'Proceedings of the Intern.Congr.Arom. Medicinal Plants and Essential Oils', Actes Editions, Rabat, Morocco, 379-384 **(1997)**.