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Chromatographic Analysis and Anthelmintic Activity of The Seed Oil of *Caesalpinia crista*

AMITA SINGHAL
Department of Chemistry, M.L.C. Government Girls Postgraduate College
Khandwa-450 001, India
E-mail: manku_singhal@yahoo.co.in

The seed oil obtained from the seeds of *Caesalpinia crista* is analyzed for its chemical composition and anthelmintic activity.

Key Words: Chromatography, Anthelmintic activity, *Caesalpinia crista*.

INTRODUCTION

The fatty acid composition of *Caesalpinia crista* seed oil has been studied by GLC, TLC and PC. The main compound being identified is methyl dodec-9-enoate. Palmitate, oleate and linoleate are also found in good quantity. The oil has almost similar anthelmintic activity as compared to standard drug piperazine phosphate against earthworm.

EXPERIMENTAL

The seeds were collected from the area of Khandwa city, shade dried, powdered and extracted with petroleum ether (60-80ºC) for 72 h in soxhlet apparatus. Removal of solvent afforded an oil (18.25%). The oil was characterized by standard IUPAC method. It was separated into saponifiable and unsaponifiable matter by ethanolic KOH method. Methyl esters were prepared by sodium methoxide method[1-4]. These methyl esters were converted into potassium hydroxamates derivative by standard method using hydroxylamine hydrochloride[5,6].

Thin layer chromatography of methyl esters: The TLC[7,8] of fatty acids, their methyl esters and potassium hydroxamates were carried out on cellulose plate treated with 10% paraffin oil solution in acetone. The solvent was 85% glacial acetic acid. The plates could not well resolved because of random overlapping of bands and tailing due to the presence of a number of components and their critical pairs. Therefore, argentation TLC[9] and reversed phase TLC[10] were carried out.

Argentation TLC of methyl esters was carried out on 12 % silver nitrate impregnated silica gel plates using petroleum ether/ether(12/8, v/v) as solvent system, spraying with 2,7-dichlorofluoresin followed by detection in UV light indicated the presence of unsaturated acids.

The reversed phase TLC was carried out on activated plates impregnated with 10% liquid paraffin in petroleum ether and developed with nitromethane /acetonitrile/acetic acid (75/10/10, v/v) as solvent system at room temperature. The spots were detected by spraying with solution of $FeCl_3$ and 0.1 M sodium molybdate solution kept at 110ºC for 3-5 min.

Paper chromatography of free fatty acids, methyl/esters and potassium hydroxamates: Whatman chromatographic paper No.1 was impregnated with 10% paraffin oil in acetone. The free fatty acids, their methyl esters and potassium hydroxamates derivatives were spotted on paper sheets. Ascending techniques was adopted for development using solvent system glacial acetic acid: water (8.5:1.5). Chromatograms were dried in air, heated at 120ºC for 2 h. Copper acetate rubeanic acid reagent was used as spraying solution, for locating the free fatty acids and their hydroxamates. Methyl esters were located by sprayed with hydroxylamine and further sprayed with acidified ferric chloride solution[11].

Gas liquid chromatography: The fatty acid methyl esters of *Caesalpinia crista* seed oil were injected into gas chromatograph on 10 % DEGS column, analyzed on Pyeunicam Series 204 gas chromatograph, equipped with flame ionization detector and a glass column of length 6' and 1/4" diameter. The temperature of injection part and detector were 170 and 300ºC, respectively, ultrapure nitrogen gas was used as carrier at a flow rate of 35 mL/min. The speed of chart was 30"/h.

Anthelmintic activity

Qualititative *in vitro* anthelmintic evaluation of the extracts was done using earthworms and adopting the techniques described by Watkins[12]. Powdered seeds of *Caesalpinia crista* were extracted with different solvents *viz*. petroleum ether (60-80ºC), benzene and alcohol serially. Removal of solvent from the extracts was done under reduced pressure to yield oily mass.

For the present study 4 and 2 % solution of all the test samples (different extracts) and standard anthelmintic drug piperazine phosphate were made in ethylene glycol[13-15].

25 mL of normal saline solution and 2 mL of test solution in ethylene glycol was transferred to the petri dishes of 4″ diameter. Two earthworms of same size were washed with normal saline solution and placed in each petri dish. The movement of earthworm were stimulated and became more marked. Therefore, they became progressively sluggish until death supervened. The same experiment was also performed with standard drug (piperazine phosphate) under the same conditions. The experiments were performed in duplicate and average paralytic time and lethal time in minutes was noted. To ascertain the death of motion less worms, one or more worms were frequently transferred to hot water at 50ºC, which stimulate and induced movement in the worms of alive.

RESULTS AND DISCUSSION

The obtained oil has the following characteristics: oil content (18.25%), colour (pale yellow), moisture (8.21 %), ash content (1.52 %), specific gravity (0.9116 %), refractive index (1.4816), viscosity (12.87 poise), acid value (5.53), saponification value (190.52), ester value (184.99) and iodine value (96.17).

The yield of oil, the specific gravity and saponification value are in agreement with those of linseed and tung oil, but not enough for commercial use in varnish and paint industries. The high iodine value indicates a high proportion of unsaturated acids, but its use as a transformer oil to the high viscosity.

PC, TLC and GLC analyses showed the presence of 16 compounds out of which only 2 compounds were not identified. The identified methyl esters were methyl capriate (1.52), caproliate (10.10), undecylate (3.56), dodec-9-enoate (16.84), myristate (2.77), tetradec-9-enoate (0.93), pentadecanoate (1.80), palmitate (13.27), 7-palmitoleate (10.26), stearate (7.56), oleate (12.33), linoleate (11.54) and arachidate (1.78%).

Godbole[14] have reported higher unsaturated fatty acids *viz.* oleic and linoleic acids as the major constituents and the percentage of linoleic acid was very high, which have not been found in present studies, but the lower unsaturated acids like caprolic acid, dodec 9-enoic acid and tetra dec-9-enoic acid were identified by us but they were not reported previously from *Caesalpinia crista* seed oil. Capriate, undecylate, myristate and pentadecanoate were also reported first time in present studies.

Thus in the seed oil of *Caesalpinia crista* the percentage of unsaturated fatty acid was 65% while 35% saturated acids were present. The peak at 1.71 and 2.70 min could not be identified, it may due to the branched chain or isomerized methyl esters.

Table-1 showed *in vitro* anthelmintic activity of the different extracts of *Caesalpinia crista* seeds. The results indicated that the anthelmintic activity of petroleum ether extract (oil) (taking only lethal time into account for discussion) was good and lethal time was 15.3 and 22.1 min at 4 and 2% concentration, respectively. The anthelmintic property was moderate in benzene extract and the value of lethal time at 4 % concentration range was 18.0 min and at 2 % concentration was 24.5 min. The alcoholic extract of *Caesalpinia crista* showed minimum activity and value of the lethal time at 4 % concentration range was 34.1 min and 2 % concentration range was 44.5 min.

As compared to standard drug piperazine phosphate the value of lethal time at 4 and 2% concentration range were 12.6 and 21.7 min which is almost similar to the value of *Caesalpinia crista* oil (petroleum ether extract).

TABLE-1
IN-VITRO ANTHELMINTICS ACTIVITY OF THE DIFFERENT EXTRACTS OF *Caesalpinia crista* SEEDS

Name of the seed extracts	Concentration			
	4 % in (EG)*		2 % in (EG)*	
	Paralytic time (min)	Lethal time (min)	Paralytic time (min)	Lethal time (min)
Petroleum Ether extract	2.2	15.3	2.5	22.1
Benzene extract	1.9	18.0	3.2	24.5
Alcoholic Extract	4.3	34.1	4.5	44.5
Piperazine phosphate	3.2	12.6	3.4	21.7

*The control experiment showed the use of solvet ethylene glycol (EG) did not show any anthelmintic activity.

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