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RESEARCH PAPER

Antidermatophytic active polyphenolic compound (*hexadecahydro-17-(2,5-dihyro-5-oxofuran-3-yl)-3,5,14-trihydroxy-13-methyl-ih- cyclopenta[a]phenanthrene-10-carbaldehyde*) from *Corchorus olitorius* L.

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Abstract

Corchorus olitorius L. is a renowned cultivated plant in India and worldwide, it is belongs to the Malvaceae family. Commonly termed nalta jute and Janumu plant. On the basis of traditional wisdom, the plant phytochemical and antidermatophytic studies were carried out. In the current work, a novel antidermatophytic polyphenolic potential flavonoid was isolated from 10 solvent ratios of column chromatography using methanolic seed extract. It was revealed that the isolated polyphenolic compound "Hexadecahydro-17-(2,5-dihyro-5-oxofuran-3-yl)-3,5,14-trihydroxy-13-methyl-iH- cyclopenta[a]phenanthrene-10-carbaldehyde" have confirmed against *M. gypsum* and *T. rubrum*. The widely prescribed antifungal Ketoconazole has MIC values of 0.1 mg ml-1 against *T. rubrum* and *M. gypseum*, compared to the isolated molecule's range of 0.12 mg ml-1. By analyzing the UV, LCMS, HPLC, NMR spectrums data, it was characterized the antidermatophytic targeted molecule.

Keywords: *Corchorus olitorius* L., Antidermatophytic, Flavonoids, polyphenolic compound, Seed methanolic extract, MIC.

Introduction

The therapeutic values of *C. olitorius* L. are enormous. The material is referred to as "nalita." Olitoriside injections significantly reduce cardiac insufficiencies and don't have any cumulative effects, thus they can take the place of strophanthin. Deobstruent, diuretic, lactagogue, and purgative tonic are some of its uses. A traditional treatment for aches and pains, enteritis, fever, diarrhea, pectoral pains, and tumours is tussah jute (Duke and Wain, 1981). The leaves are used in Ayurveda to treat tumours, piles, ascites, and discomfort. The leaves are also used in other places to treat gonorrhoea, fever, dysuria, and cystitis. According to legend, the cold infusion increases appetite and vigour (Duke 1981).

Due to the presence of phytochemical substances in the seed, it has shown rich therapeutic ideals (Taoging Zhou et al. 2009). The seed extracts were shown the hypoglycemic effects due to the presence of flavanoids, alkaloids, and saponins (George Francis *et al.* 2002). In accordance with Adeneye and Adeyemi (2009) *Hunteria umbellate's* aqueous seed extract has hypoglycaemic effects in normoglycaemic, glucose- and nicotine-inducing situations because to the phytochemicals like alkaloids, flavonoids, tannins, and glycosides it contains (Kaku Nakagawa *et al.* 2004). This indicates that the hypoglycemic effects of the seed extract of *C. olitorius* have reported hypoglycaemic effect with a variety of phytochemicals (Adeneye *et al.* 2009).

The leaves of *C. olitorius* L. comprises protein, fat, carbohydrate, fibre, ash, calcium, potassium, iron, salt, phosphorus, beta-carotene, thiamine, riboflavin, niacin, ascorbic acid, etc. Seventeen active nutritional components

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have been found in the leaves, and Oxydase and chlorogenic acid are also reported from the leaves (Calleja, 2010). The folic acid level reports substantially higher than that of other folacin-rich plants, at about 800 micrograms per 100g (about 75% moisture) or about 3200 micrograms on a moisture-free basis recorded by Chen and Saad, (1981).

The leafy green vegetable are also good source of beta-carotene, iron, calcium, and vitamin A, C, E, which are all essential for healthy red blood cells, smooth, clear skin, robust immune system function and quick wound healing, free radicals (Chen and Saad 1981). It is an essential green leafy vegetable in several tropical countries, including India (Samra *et al.* 2007). The matured leaves are used as vegetable and fodder (Zakaria *et al.* 2006).

Surprisingly, the seed extract of *C. olitorius* L. used in folk medicine to treat gonorrhoea, pain, fever, and tumours (Ndlovuand Afolayan 2008). The plant aerial part having great source of fiber, minerals like calcium and iron, vitamins A and C, and minerals. Because of its high concentrations of carotenoids, vitamins B, B_2, C, and E, and minerals, it is supposedly a popular vegetable in Japanish dish. In South East Asia, its leaves and roots are consumed as herbal remedies (Zeghichi *et al*. 2003). Aqueous extracts of *C. olitorius* seeds revealed to have anti-inflammatory, anti-pyretic, and anti-nociceptive effects on both the peripheral and central nervous systems (Zakaria *et al.* 2006). Preliminary investigation has revealed that the seeds contains purgative effect, the moderate ratio of cardio glycosides also reported and that the methanol extract of the seeds have recored wide range of antibacterial activity (Pal *et al.* 2006).

Screening of natural products from plants in a search for a new antimicrobial agent that would be active against organisms is the need of the hour. The leaves of *C. olitorius* L. was reported to have hypoglycaemic effect and high antibacterial activity (Adegoke and Adebayo-Tayo 2009). The seed protein enriched diet was found to increase rats body weight (S. Laskar *et al.* 1986). The seeds were found to contain reasonable percentage of biologically active cardiac principals (Sharaf and Negm 1969). The plant stem is a source of jute fibre and folkloric uses includes, seeds for purgative, leaves for dysentery, fever, gonorrhea and demulcent (Watt 1962).

The seed oil have shown antibacterial activity against *P. aerugenosa,* which causes bladder inflammation, *K. pneumonia,* which reason for pneumonia, *S. aureus,* which effects food poisoning, *S. typhimurium,* which source of typhoid fever and *B. cereus,* which contaminates eye, food spoilage, and food borne intoxication (Nester *et al.* 2004).

However, the results of the seed oil have reported antibacterial activity as claims made by Burt (2004) and Karaman *et al.* (2003) that the gram positive bacteria are more sensitive than gram negative bacteria. The results of Doughari *et al*. (2007), who indicated that pawpaw root extracts show greater antibacterial action on Gram negative than Gram positive bacteria, are consistent with this. The nature and combinations of phyto-compounds found in the essential oil and the intrinsic tolerance of the microorganisms may both contribute to the various extents of susceptibilities of the bacterial isolates.

Although having reports from other aspects, there are no prior reports on phytochemical analysis and antidermatophytic activity of the seed extract so that the current report is aimed to perform the antidermatophytic phytochemical active compound isolation, characterization, and structural analysis confirmations.

Materials and methods

Plant material collection

The plant material were collected from Hyderabad, Karnataka region and placed in a new bag before being transported to the lab. The materials were initially cleaned with condensed water to remove pollutants and dried on sheets in a laboratory at 37 °C for a week before the plant was verified and submitted to the Department of Botany. Gulbarga University for the voucher number receipt at herbarium centre for authentication (Seetharam 2000).

Crude drug extraction by hot extraction by soxhlet apparatus (Horborne 1998)

In 25 g of the fine powder of the selected plant material was weighed and followed successive extraction for 48 hours. The crude extracts were collected and stored in air tight bottles further use.

Preparation of extract dilution series

The crude extract 400 mg dissolved in 10 ml DMSO for antidermatophytic activity, the concentration is considered as 40 mg/ml. Like the same the various concentrations of extract stock prepared i.e., , 20 mg/ml, 10 mg/ml, 05 mg/ml, 2.5 mg/ml. 1.25 mg/ml. 0.62 mg/ml. . Here the only DMSO without crude extract used as negative control. The standard and positive control used fluconzole against fungi and against bacteria streptomycin 100 µg/ml used for test.

Microbes used in the studies

The effectiveness of dermatophytes such *Microsporum gypseum, Trichophyton rubrum, Trichophyton tonsurans, Aspergillus flavus, and Candida albicans* were investigated in the current study. All the test strains were obtained from the Department of Microbiology of Maha Devappa Rampure Therapeutic College, Karnataka, MTCC of Chandigar, India. As usual, bacterial cultures were grown in NB at 37°C and kept on NA inoculation, whereas fungal strains were cultured in PDA at 28°C and kept on PDA slants at 4°C.

Microbial inoculums preparation

Dermatophytes had been growing on SDA for a week before spores were produced by flooding with 0.85% briny. After the larger particles had settled in the test tube, the

supernatant was taken out, and the conidia present were counted using a hemocytometer. A tenfold dilution was carried out. Nine millilitres of ordinary saline solution were placed in each of five test tubes. One cc of spore suspension was mixed into the first test tube while maintaining aseptic conditions. To the second test tube, which contained 9 ml of ordinary saline solution, homogenised pieces of the first test tube's solution were added.

The procedure was repeated for the sixth test tube. In each case, pipettes were used that had been sterilised. 0.1 ml of suspension from each test tube was added to SDA petri plates that had been cleaned for each dilution. The three duplicates of each dilution were stored. The SDA-seeded petri plates were subjected to hemocytometer counts. An average of 3 petri plates were collected for each case. The tested inoculum range of 1.5x105 spores/ml was modified (Santos 2006).

Anti-dermatophytic activity

Using the agar well diffusion method to determine the MIC (Magaldi, 2004)

The experiment was carried out using the cup well diffusion technique. PDA medium was placed in clean petri dishes, A fungal lawn was prepared using a five-day-old culture strain. The different fungal strains each separately turbidity threshold of 0.5 Mac. Farland standards are 108 CFU/ml, suspended in a 0.85% NaCl saline solution. One cc of the test strains was spread over the medium using a sterilized glass spreader in already poured petri plates. Using a flamed sterile borer, 4 mm-diameter wells were punctured in the culture medium.

The required concentrations of serially diluted extracts (0.6, 1.2, 2.5, 5, 10, 20 and 40mg/ml) were filled in the wells with well labeled. Incubate antibacterial plates at 37°C and the antidermatophytic plated at 28°C. After 48 hours, the plates were observe, calculated and recorded the inhibitory zones.

Broth Dilution Assay for MIC Determination (NCCLS, 1997)

The minimal inhibitory concentration of a crude extract was determined using a broth dilution test. The medium, which was produced through serial dilution, contains different concentrations of plant extract, ranging from 100 mg to 1 mg per ml (10-1 dilution). After culture inoculation, the test tubes were incubated for 72 hours at 28°C. The MIC of each extract were calculated, the turbidity of the inoculum was detected by spectro-photometer at 520 nm and comparing the results with the non-inoculated broth used as a blank. Control was used without extract. The experiment carried out in the conformity with NCCLS guidelines, which are now known as CLSI guidelines (Ogunwande, I.A. *et al.* 2011).

Secondary metabolite preliminary screening tests

The plant extracts used for preliminary testing using standard techniques for the detection of secondary metabolites (Harborne, 1998). The test solution was created by combining the appropriate solvent with 500 mg of each extract in 100 ml, which was then filtered through Whattman filter paper No. 1. The filtrates were consequently used as test solutions for the successive screening tests.

Isolation of active fraction from selected plant extract using column chromatography (CC) followed standard Wilson and Walker (1995) method.

For the column chromatography the column i.e., 45 cm portion of a clean, dry, 500 ml volume column (made by Vinsel) is filled with a slurry of silica gel-H with a mesh size of 60–120 (hi-media, Mumbai). Hexane is then used to fill the remaining space. The stationary phase was making it possible into the column to minimize air bubbles. It was then cycled twice with a solvent system that contains analytical grade hexane to generate an airtight and compact column (Sd-fine Chem., India).

Loading of the active extract into the column with successive methanol extract about 400 mg added into the column.

Collections of column elucidated fractions with two combinations of the solvent ratio were followed from low to high polarity. The column elucidated fractions were collected about 100 ml at a time, in a succession of conical flasks as they come out from the column (Borosil, India). Among the 10 fractions again follow the TLC of each fraction meanwhile the fractions were performed antidermatophytic activity was performed. These fractions were used to perform thin layer chromatography. Similar fractions were collected and vacuum-condensed to isolate the active compound based on the TLC results, which tested against dermatophytic fungus, the active fraction separated and purified with followed further procedures.

Isolation, purification of antidermatophytic compound by using Preparative Thin Layer Chromatography (PTLC) The active compound isolated and refined using the column chromatography fractions and colour intensity band width as determined by PTLC The preparation of PTLC plates were prepared, as stated earlier, 20x10 cm glass plates containing 1mm thick neutral silica gel were used for compound separation. The loading and development of a capillary tube was used for loads with 500 l of the active cc collected fraction, and it was left to dry. These plates were placed in a saturated, developing solvent-mixture-filled chromatographic chamber.

After run of the column followed the PTLC, the fractions ratio, nature recorded and scraped the fraction and used for the detection of antidermatophytic activity at every stage of collection. On the basis of active fraction will be used further detections of the phytochemical, structural analysis. The observations of chromatograms were meticulously marked with a needle while being examined under wave length (UV254 and UV365 nm).

Table 1: Antidermatophytic activities of methanolic seed extract of *Corchorus olitorius* (Well diffusion technique).

Fungal strains	Different conc. (mg/ml) of crude and inhibition zone in mm								
	40	20	10	5	2.5	1.25	0.62	Control (DMSO)	Standard (Ketoconazole)
T. rubrum	11. 33±1.52	10. 00±0.00	09. 33±1.52	07. 33±1.52	06. 33±1.52	05. 00±0.00	_	-	26. 66±0.57
M .gypseum	12. 33±1.52	10. 00±0.00	09. 66±0.57	08. 66±1.15	06. 33±1.52	05. 00±0.00	_	-	23. 66±1.15
C .albicans	15. 00±0.00	11. 33±1.52	09. 33±1.52	08. 00±0.00	07. 00±0.00	06. 66±0.57	_	-	23. 33±1.52
T.tonsurans	13. 00±0.00	10. 66±0.57	09. 66±1.15	08. 66±1.15	06. 33±1.52	05. 66±1.15	-	-	18. 00±0.00
A. flavus	10. 00±0.00	09. 66±1.15	07. 66±0.57	06. 66±1.15	05. 66±1.15	-	-	-	22. 00±0.00

T. rubrum: Trichophyton rubrum, M. gypseum: Microsporum gypseum, *C .albicans: Candida albicans, T.tonsurans: Trichophyton tonsurans*, *A. flavus: Aspergillus flavus,* Negative control: DMSO N, N- Dimethyl Formamide, Standard: Ketoconazole (Positive control).

Table 2: Antibacterial activity of methanolic seed extract of *Corchorus olitorius* (Well diffusion technique).

Bacterial strains	Different conc. (mg/ml) of crude and inhibition zone in mm								
	40	20	10	05	2.5	1.25	0.62	Control (DMSO)	Standard (Streptomycin)
E. coli	16. 00±0.00	13. 66±0.57	11. 66±1.15	10. 00±0.00	09. 66±0.57	07. 66±0.57	06. 66±0.57	-	24. 66±0.57
B. subtilis	16. 66±0.57	15. 66±0.57	14. 66±1.15	13. 66±1.15	12. 66±1.15	11. 66±0.57	10. 66±0.57	-	26. 66±0.57
S. marcescens	19. 66±1.15	18. 66±0.57	16. 00±0.00	14. 66±0.57	12. 66±0.57	09. 66±0.57	_	-	30. 66±1.15
S. aureus	15. 66±1.15	13. 00±0.00	11. 00±0.00	10. 66±1.15	09. 00±0.00	08. 00±0.00	05. 00±0.00	-	28. 00±0.00
P. aeruginosa	17. 66±1.15	15. 66±0.57	13. 66±0.57	12. 00±0.00	10. 66±0.57	09. 66±0.57	07. 00±0.00	-	28. 00±0.00
B. brevis	13. 66±0.57	11. 66±0.57	10. 00±0.00	08. 66±0.57	07. 00±0.00	06. 00±0.00	_	-	24. 66±0.57

E. coli: Escherichia coli, B. subtilis: Bacillus subtilis, S. marcescens: Serratia marcescens, S. aureus: Staphylococcus aureus, P. aeruginosa: Psudomonas aeruginosa,

B. brevis: Brevibacillus brevis, Negative control: DMSO N, N- Dimethyl Formamide, Standard: Streptomycin sulphate (Positive control).

Table 3: Preliminary screening of secondary metabolites in *Corchorus olitorius* L. seed.

Secondary metabolites	*Name of the test*	*PE*	*CHCL3*	*EtOH*	*Methanol*
Alkaloids	Mayers test	_	_	_	+
	Dragendroff's test	+	_	+	+
	Wagner's test	+	_	_	_
Phenol	Hot water test	_	_	_	_
	Ferric chloride test	_	_	+	+
	Ellagic acid test	_	_	_	+
Flavonoids	Ferric chloride test	_	_	+	+
	Lead acetate test	_	+	+	+
	Shinoda test	_	_	+	+
	NaOH test	_	+	+	+
Tannins	Gelatin test	_	_	+	+
Triterpenoids	Salkowski's test	_	_	+	+
	Libermann-Burchard test	_	_	+	+
Steroids	Salkowski's test	_	_	+	+
	Libermann-Burchard test	_	_	+	+
Saponins	Foam test	_	_	_	+
Glycosides	Keller-Killiani test	_	_	+	+
	Conc. H2So4 test	_	_	_	_
	Molisch's test	_	_	_	_
	Glycoside test	_	_	_	_

For the Purification of compound the combination relevant solvents, such as chloroform and methanol (90:10). 5000 rpm centrifuged for 10 minutes to get the purified compound-containing supernatant. The pellet was centrifuged after being once more re-suspended in the solvent combination. The combined supernatants were vacuum-condensed to dryness at 40°C, and the compound was then retained at 4°C in a refrigerated vial with a tight screw closure of a 5 ml capacity (Hi-Media Lab., Mumbai).

The antidermatophytic active compound studied with LCMS, ^{H}NMR, ^{C}NMR, HPLC for the structural analysis. The compound physical, chemical, nature and antidermatophytic

Table 4: Qualitative separation of secondary metabolites from *Corchorus olitorius*

Secondary metabolites	*No of bands*	*hRf values*	*Colour of the bands*
Phenols	4	17.39	Brown
		23.91	Light brown
		54.35	Yellow
		76.08	Smoke brown
Flavonoids	2	61.2	Brownish
		73.46	Greenish brown
Alkaloids	2	14.21	Light Brownish
		21.57	Slight Yellowish

analysis with Minimum Inhibitory Concentrations will be performed. The antidermatophytic IUPAC name, structure will be plated.

Results and discussion

The plant material of *Corchorus olitorius* L. species collected from Manvi thaluk of Raichur District of Karnataka state, the herbarium was deposited at Department of Botany, Gulbarga University., the voucher number HGUG:2347 allotted. The plant seeds methanolic successive extract was collected by using hot extraction with soxhlet apparatus. The crude of the extract was used for the antidermatophytic activity. At initially screening against skin pathogens was performed. On the basis of the results further the phytochemical analysis and isolation procedures were followed.

Antidermatophytic activity and minimum inhibitory concentrations of the crude extract

The current experiment examined the antifungal and antibacterial activities of methanol seed extract of *Corchorus olitorius* against five fungus species and six bacterium species. The median values of the three observations are shown in Table 1 & 2. Plate 1.

The methanol seed extract inhibited *Trichophyton tonsurans* (12.00±0.00mm), *Microsporum gypseum* (12.33±1.52 mm), and *Candida albicans* to a maximum of 15.00 0.00 mm at a concentration of 40 mg/ml. The test fungi utilised to identify the lowest inhibitory doses were *Aspergillus flavus* (10.00±0.00mm) and *Trichophyton rubrum* (11.33±1.52mm). The results are displayed in Figure 1. The MIC for *M. gypseum* was 0.62 mg/ml, while *T. rubrum, C. albicans,* and *T. tonsurans* had concentrations of 1.25 mg/ml conc. Despite the fact that the MIC for *A. flavus* was found to be 2.5 mg/ml conc.

Serratia marcescens was the organism with the largest zone of inhibition by the methanol seed extract at 40 mg/ml conc., followed by *Psudomonas aeruginosa* (17.66±1.15 mm), *Bacillus subtilis* (16.66±0.57 mm), *Escherichia coli* (16.00±0.00 mm), *Staphylococcus aureus* (15.66±1.15 mm), and *Brevi bacillus.*

The information regarding the test bacterium's minimal inhibitory concentration is shown in Figure 1. *E. coli, B. subtilis, S. aureus*, and *P. aeruginosa* all had conc. MICs of 0.62 mg/ml. It was established that the MIC for *S. marcescens* and *B. brevis* was 1.25 mg/ml conc. The DMSO-based negative control failed to inhibit any of the tested bacterial or fungal species. *Streptomycin exhibits* an inhibition zone of 24.66 ±1.15 mm when used as a standard against bacteria, but when used as a standard against fungi, ketoconazole at conc.5 mg/ml exhibits antifungal activity of 18.66±0.57 mm.

Preliminary screening for the occurrences of secondary metabolites

The presence of various secondary metabolites, such as alkaloids, phenol, flavonoids, tannins, triterpenes, steroids, saponins, and glycosides, were qualitatively screened for the crude successive extract of the seed, which included petroleum ether, chloroform, ethyl-acetate, and methanol extracts.

Table 5: Isolation of compound fractions through column chromatography.

Sl no	*Mobile phase*	*Ratio of mobile phase*	*Number of fractions*	*Colour of the extract*	*Nature of the extract*	*Weight of the extract*	*Antidermatophytic (T.rubrum) activity*
1	n-Hexane	100:00	1	Light Yellow	Creamy	0.15	09.00
2	n-Hexane : Ethyl acetate	90:10	3	Yellow	Waxy	0.33	05.00
3	n-Hexane : Ethyl acetate	80:20	2	Light yellow	Waxy	0.26	07.00
4	n-Hexane : Ethyl acetate	70:30	2	White precipitate slight yellow	Amorphous	0.31	11.00
5	n-Hexane : Ethyl acetate	60:40	1	Transparent	Amorphous	0.18	06.00
6	n-Hexane : Ethyl acetate	50:50	2	Light brown	Solid	0.36	05.00
7	n-Hexane : Ethyl acetate	40:60	2	Yellowish	Solid	0.11	-
8	n-Hexane : Ethyl acetate	30:70	2	Light brownish	Solid	0.39	06.00
9	n-Hexane : Ethyl acetate	20:80	4	greenish	Solid	1.20	-
10	n-Hexane : Ethyl acetate	10:90	3	Light green	Solid	0.83	10.00
11	n-Hexane : Ethyl acetate	00:100	1	Yellow	Semi-Solid	0.66	04.00

Table 6: Antidermatophytic activity & minimum inhibitory concentration of isolated compound COR-1.

Compound code	*Test strain*	*Inhibition zone in mm & in different conc. of compound*					
		01mg^{-1}	*0.5mg^{-1}*	*0.25mg^{-1}*	*0.12mg^{-1}*	*Control*	*Standard(K) 01mg^{-1}*
CR-1	*T.rubrum*	16. 00±0.00	13. 66±1.15	10. 66±1.15	08. 66±0.57	_	18. 00±0.00
	M. gypseum	14. 66±1.15	11. 00±0.00	07. 33±1.52	05. 00±1.00	_	21. 66±1.15

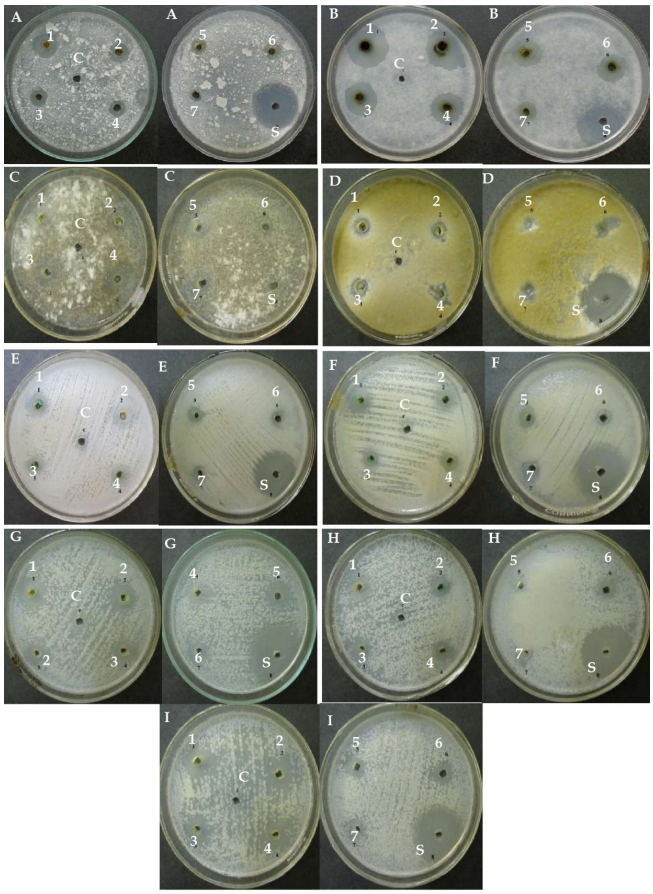


Plate 1: Antidermatophytic activity of methanolic seed extract of Corchorus olitorius (Well diffusion technique).

A: *Trichophyton rubrum*, B: *Microsporum gypseum*, C: *Trichophyton tonsurans*, D: *Aspergillus flavus* E: *Candida albicans*, F: *Escherichia coli*, G: *Bacillus subtilis*, H: *Staphylococcus aureus*, I: *Psudomonas aeruginosa*, 1=40 mg/ml, 2=20 mg/ml, 3=10 mg/ml,4=5 mg/ml, 5=2.5 mg/ml, 6=1.25 mg/ml, 7=0.62 mg/ml, C=Negative control: DMF N, N- Dimethyl Formamide, 8=Standard: Ketoconazole (Positive control against fungi),Streptomycin sulphate (Positive control against bacteria).

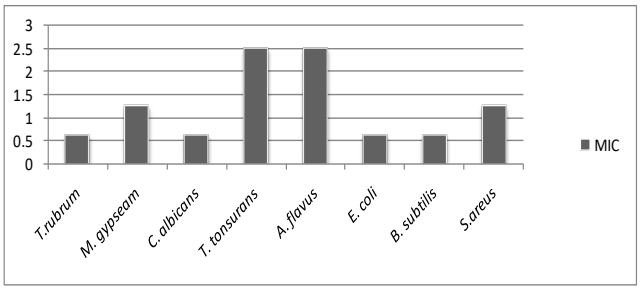


Figure 1: Minimum inhibitory concentrations of methanolic seed extract of *Corchorus olitorius* L. against test strains.

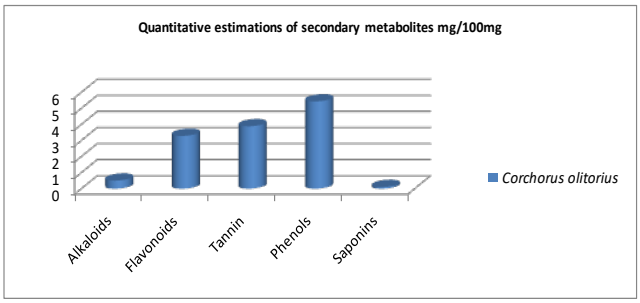


Figure 2: Quantitative estimations of secondary metabolites in seeds of *Corchorus olitorius* L. in mg/100mg.

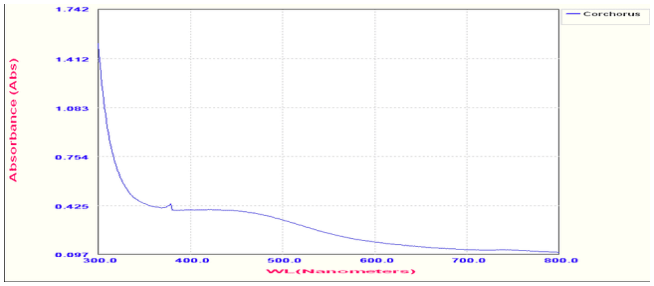


Figure 3: UV-Spectrum of CR-1

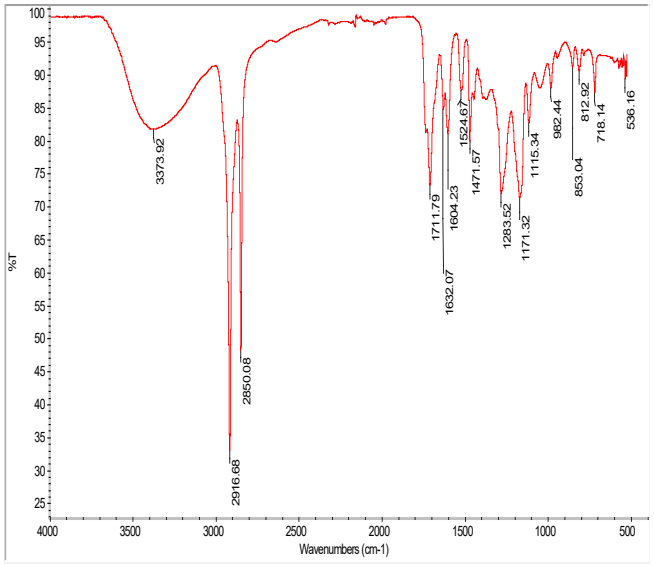


Figure 4: Infrared spectrum (IR) of CR-1

The methanol extract produced positive findings in the initial alkaloids testing using Mayers and Dragendroff's reagents. These extracts produced a creamy white precipitate when used with Mayers reagent and an orange red precipitate when used with Dragendroff's reagent. Nevertheless, the dragendroff and wagner reagents had a favourable reaction with the petroleum ether extract. The ethyl acetate extract responded favourably to Dragendroff's reagent. The chloroform extract, on the other hand, failed all three tests.

The methanolic seed extract's reaction to the phenols test was positive for both the ferric chloride test and the ellagic acid test. The intersection of the dipped and un-dipped portion was not visible during the hot water test. Test using ferric chloride reveals a favourable response from ethyl acetate extract. The petroleum extracts and chloroform are not displayed for any of the three tests.

The ethyl acetate and methanolic seed extracts responded positively to the flavonoids test employing ferric chloride, lead

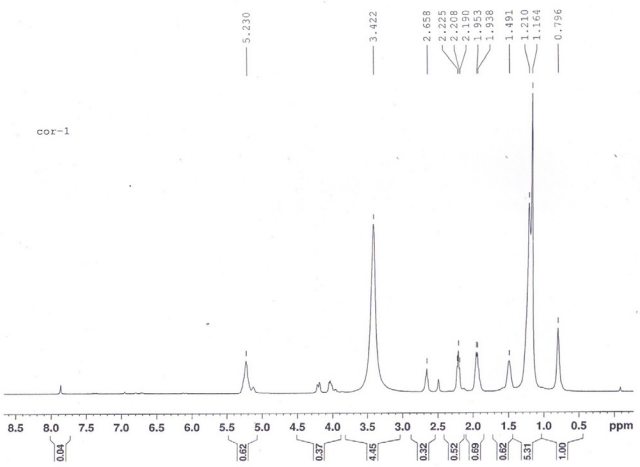


Figure 5: ^{H}NMR Spectrum (^{H}NMR) of CR-1

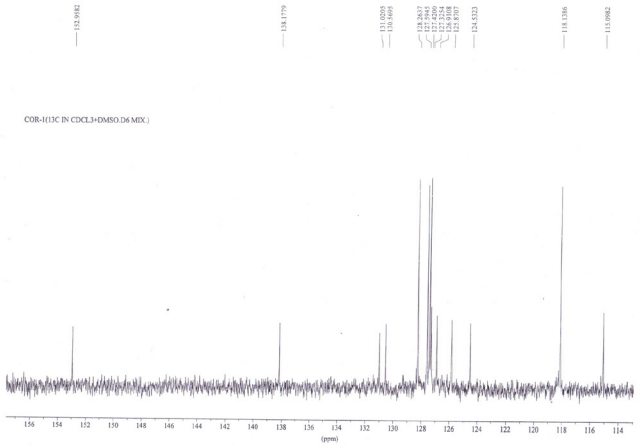


Figure 6: CNMR Spectrum (CNMR) of CR-1

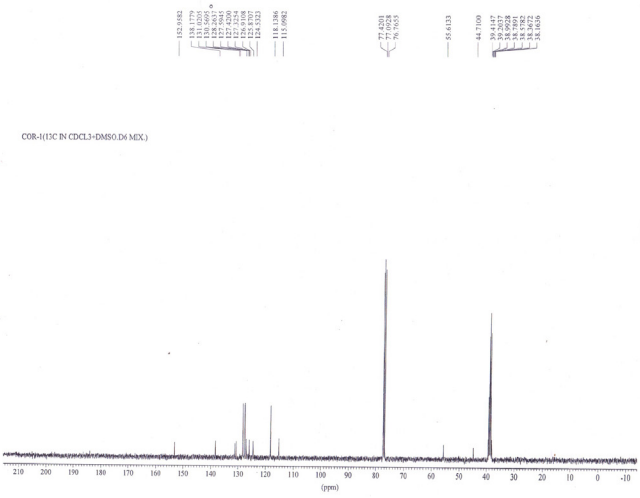


Figure 6.1: CNMR Spectrum (CNMR) of CR-1

acetate, shinoda, and NaOH, demonstrating the presence of flavonoids. While the chloroform extract test findings for lead acetate and NaOH are favorable. But none of the four experiments went well for the petroleum-ether extract.

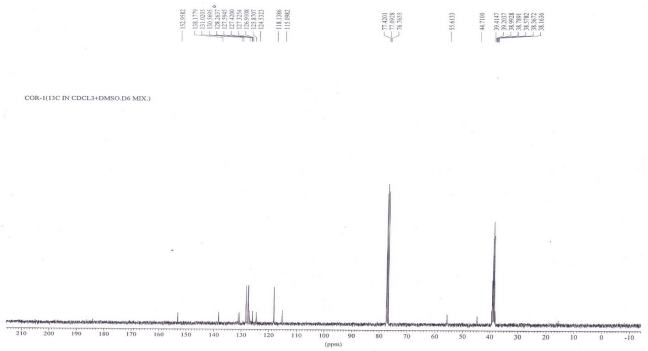


Figure 6.2: ^{C}NMR Spectrum (^{C}NMR) of CR-1

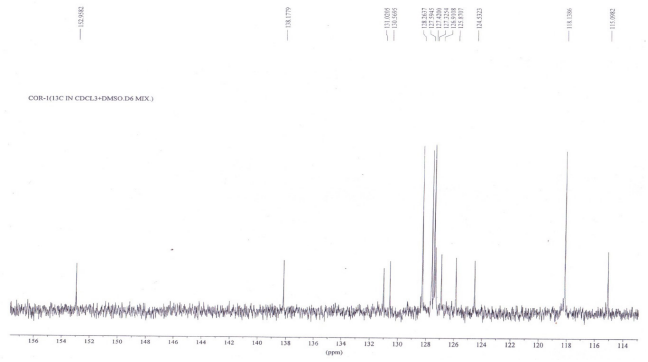


Figure 7: ^{C}NMR Spectrum (^{C}NMR) of CR-1

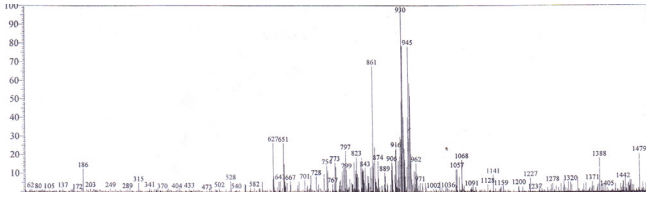
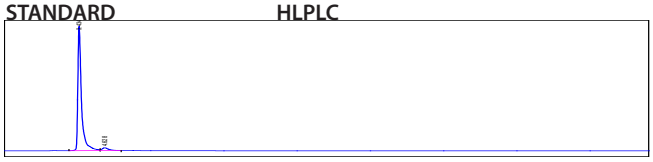


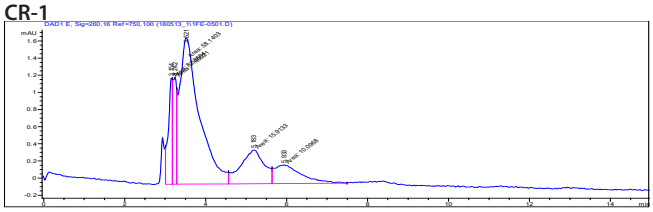
Figure 8: LCMS Spectrum (LCMS) of CR-1 (PEAK 404 m/z)

STANDARD **HLPLC**



Sl.No.	Time	Area	Height	Width	Area%	Symmetry
1	3.434	18585.7	1559.4	0.175	96.074	0.472
2	4.628	759.6	35.3	0.3151	3.926	0.594

CR-1



Sl. No.	Time	Area	Height	Width	Area%	Symmetry
1	3.154	8.5	1.2	0.1149	8.512	2.922
2	3.242	7.6	1.2	0.1016	7.578	1.349
3	3.521	58.1	1.7	0.5677	57.974	0.476
4	5.183	15.9	3.90E-01	0.676	15.868	0
5	5.933	10.1	2.20E-01	0.7756	10.068	0.506

Figure 9: HPLC Profile of CR-1

Tannins test responses for the seed extracts in chloroform, ethyl acetate, and methanol were positive for the gelatin test result. This implies the presence of tannin. However, the gelatin test did not go well for the petroleum-ether extract.

Triterpenes test response shown for the Salkowski's method was well-received by the ethyl-acetate and methanol extracts, with Libermann-Burchard adding the existence of triterpenes. The petroleum-ether and chloroform extracts, in comparison, failed both tests.

Salkowski responded favorably to extracts of ethyl acetate and methanol in the steroid test, and Libermann-Burchard confirmed the presence of steroids. However, the petroleum ether and chloroform extracts produced failed both tests.

When the methanol extract responded favourably to the tests with foam saponins, it was revealed that saponins were present in the extract.

The Kellar-Kiliani test for glycosides produced a positive result from the extracts of ethyl acetate and methanol, demonstrating the presence of glycosides. Not all of the tests are answered for four extracts.

Quantitative estimations of secondary metabolites

Five significant secondary metabolites were extracted from the dried, powdered *Corchorus olitorius* seed material and quantitatively assessed using various methods. Refer to Figure 2. The highest estimated concentration was for total phenol (5.43 mg/100 mg), which was followed by total tannins (3.88 mg/100 mg), flavonoids (3.28 mg/100 mg), total alkaloid (0.5 mg/100 mg), and total saponins (0.1 mg/100 mg).

Qualitative separation of secondary metabolites by TLC method

The following medically important systemic chemicals were separated from seed using various solvent systems and thin layer chromatography. We noted the hRf values and distinctive colours of the bands.

The separation of phenols resulted four distinct bands with corresponding hRf values of 17.39, 23.91, 54.35, and 76.08. Brown, light brown, yellow, and smoke brown were the colours of these band. (Table 4). Where the separation of flavonoin revealed two separate bands with hRf values of 61.2 and 73.46 each, which were brownish and green brown, respectively, were seen on the seed chromatogram that was created. In the alkaloids separation two distinct bands with hRf values of 14.21 for light brown and 21.57 for mild yellow could be seen on the seed's chromatogram (Table 4).

Separation of polyphenolic fractions from Corchorus olitorius seed by the Column chromatography and PTLC (Preparative thin layer chromatography)

Corchorus olitorius L. seed contains the rich source of flavonoids (Ploy phenolics are the group of flavonoids) of pharmacological significance, as shown by earlier findings of qualitative and quantitative research of polyphenolic. The literature that is currently accessible on *Corchorus* species also supports this. As a result, an attempt was made to purify some of these flavonoids fractions from the seed of *Corchorus olitorius* L. using preparative thin layer chromatography and column chromatography (PTLC) methods.

Separation of polyphenolic fractions followed by Column chromatography technique (CC)

At room temperature and pressure (26°C. 1 bar), 10 g of the crude effective extract of *C. olitorius* L. seed was separated on a Silica gel-H (60-120Mesh) column. 38 fractions totaling 100 ml each were collected after 200 ml of the column's dead volume (hexane) were discarded. The fractions 1 to 11 were obtained from the Hexane: ethyl acetate. (1) 100:00, 90:10 (2), 80:20 (3), 70:30 (4), 60:40 (5), 50:50 (6), 40:60 (7), 30:70 (8), 20:80 (9), 10:90 (10) and 00:100 (11). Due to their similarity in colour, the 23 fractions that were gathered were combined into 11 major fractions. The waxy nature fractions of fractions 2 and 3, 4 and 5, 06 to 10 (solid) brown, and 11 were found in the concentrated solutions of these fractions (semi-solid) Fractions 01 through 03 exhibits a yellow colour, fractions 04 exhibits a white precipitate with a faint yellow tint, and fractions 10 exhibit a light green colour (Table 5).

Separation of polyphenolic fractions by PTLC

Out of the four flavonoids fractions, two fractions (CR-I) were and with the macro quantity of compound possible by using the preparative thin layer approach. In addition to the TLC was used to verify the purity of these fractions using different solvent systems, which resulted in the emergence of a single separated spot for each effective fraction. The purified substance that was isolated using a chromatographic technique from the effective fraction.

The polyphenolic isolated compound Hexadecahydro-17-(2,5-dihyro-5-oxofuran-3-yl)-3,5,14-trihydroxy-13-methyl-iH- cyclopenta [a]phenanthrene-10-carbaldehyde was found to be potentially active against *T. rubrum* and *M. gypseum*. The MIC values of the isolated compounds ranged in between 0.12 mg ml^{-1} whereas MIC values of standard antifungal agents ketoconazole was 0.3 mg ml^{-1} against *T. rubrum* and *M. gypseum*.

Physico-Chemical properties & Characterization of isolated compound CR-1

The UV value showed 380 nm. The FTIR 3373 Broad peak (-OH), 2916 (Aliphatic-CH Streching), 1711(Oxo-furanyl carboxyl funeion), 1632 (CHO). The ^1H NMR values are shown i.e., δ 1.4 (s, 3H, CH_3), 1.49 (q, 1H, CH), 1.52 (q, 4H, 2xCH_2), 1.56 (t, 2H, CH_2), 1.63 (q, 1H, CH), 1.64 (q, 2H, CH_2), 1.68 (t, 2H, CH_2), 1.72 (q, 2H, CH_2), 1.75 (t, 2H, CH_2), 1.83 (d, 2H, CH_2), 2.08 (t, 2H, CH_2), 3.17 (q, 1H, CH), 2.17 (d, 1H, Pentyl-CH), 3.58 (s, 1H, OH), 3.65 (s, 2H, 2XOH), 4.9 (s, 2H, CH_2), 5.93 (S, $1H_1$, CH), 9.52 (s, 1H, CHO). The 13C NMR values are shown i.e., δ 208.2 (CHO), 174.0 (C=O), 1171 (Oxa-pyranyl-CH), 66.7, 76.0, 86.0 (3X-C-OH), 7.3.6 (Oxa-pyranyl-CH_2), 16.7 (CH_3), 20.7 to 49.7 (-CH_2). The mass confirmed the molecular formula: C_{23} H_{32} $O_{6,}$ the molecular

weight shown 404.50, m/z 404.22 (100%). The elemental analysis resulted the values shown i.e., C, 68.29, H, 7.97, O, 23.73. While calculated values shown C, 68.31, 7.92, 23.76.

Nature: crystalline, Colour: Dark brownish, hRf values: 61.25, Solvent system: n-hexane: ethyl acetate (7:3), Melting point: 190-200, Solubility: soluble in water, dilute acids and alkalies. Active fraction: n-Hexane: Ethyl acetate, 70:30, White precipitate slight yellow, Amorphous *Hexadecahydro-17-(2,5-dihyro-5-oxofuran-3-yl)-3,5,14-trihydroxy-13-methyl-iH-cyclopenta[a]phenanthrene-10-carbaldehyde*.

In the current antimicrobial activity is effective than the past reports of Sumengen *et al*. 2018 and Ilhan 2007, while Adegoke A *et al*. 2009 reported the phytochemical composition and antimicrobial using seed extracts on four bacteria's but in the present report the seed extract shown effective antibacterial activity (Soykut G 2017, Mohammed 2016). Phenolic antioxidants from the leaves past report over than results have reported using seeds (Azuma K 1999, Sarker 2018, Guzzetti 2021). A Comparative study on the chemical composition from seed and stem dry oils were reported but they were unable to concentrated seed composition (Al-Yousef 2017). The biochemistry, medicinal and food values of leaves have reported pastly, where as the current report concentrated with the similar values of seed (Islam 2013, Zeghichi 2003). Presently the poly phenolic compound characterized, partly flavonoids and cynnamoil derivatives were reported by Ola 2009.

Conclusion

According to the current isolated compound and its antidermatophytic results could be concluded that *Corchorus olitorius* seed extract are the source of biologically useful molecule. When antidermatophytic activity tested against *T. rubrum*, the active compound isolated named i.e., hexadecahydro-17-(2,5-dihydro-5-oxofuran-3-yl)-3,5,14-trihydroxy-13-methyl-iH-cyclopenta[a]phenanthrene-10-carbaldehyde, revealed remarkable activity. The results of this study confirmed that the isolated polyphenolic compound as the effective antidermatophytic molecule. The new phytochemical compound connects to the upcoming researchers. New study will be needed to completely comprehend the mode of actions and mechanism of the antidermatophytic compound at molecular level.

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