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PHYTOCHEMICAL, ANTIMICROBIAL AND TRADITIONAL USES OF LEAVES EXTRACTS OF CORCHORUS FASCICULARIS LAM.

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ABSTRACT

Phytochemical investigation of leaves of Corchorus fascicularis which includes physicochemical parameters like ash values, extractive values and moisture content. The total ash, acid insoluble ash, water soluble ash values and sulphated ash were observed to be 5.40%, 2.96%, 1.85%, 0.65% respectively. Alcohol soluble and water soluble extractive values of leaves ware observed to be 2.90%, 5.54% respectively. Phytochemical investigation of n hexane, chloroform, ethanol and water extract revealed the presence of glycosides, tannins, terpenoids, steroids, carbohydrates, alkaloids, saponins and proteins. The antimicrobial activity was determined in all extracts by using agar disc diffusion method. Extracts were effective on tested microorganisms. The antibacterial and antifungal activities of solvent extracts of Corchorus fascicularis L. were tested against one gram positive, one gram negative human pathogenic bacteria and one fungus respectively. All the extracts showed broad spectrum of inhibition by showing antibacterial effect of both bacterial strains. For isolation of compounds, the dried leaves powder of Corchorus fascicularis Lam. was subjected for cold maceration at room temperature with ethanol and subjected to column chromatography. Two compounds were isolated and purified by methanol. Mass spectrum of EEC-1 and EEC-2 showed a parent molecular ion peak at m/z 302 and 290 which corresponds to molecular formula C15H14O7 and C15H14O6. The structures were determined as Quercetin and Catechin by physical, chemical tests and spectral characterization such as Elemental analysis, Nuclear magnetic Resonance and Infrared spectrometry.

Keywords: - Quercetin, Catechin, cold maceration, ethanol extracts, Antibacterial activity Antifungal activity Corchorus fascicularis L.

INTRODUCTION

The development of medicinal plant as medicine is good way. In food producing countries to control growth of bacterial in the product is important. The most common bacteria causing food borne illness are S. aureus, E. coli and others1,2. Natural products of higher plants may give new source of antimicrobial agents with novel mechanisms of action3, 4.

Corchorus fascicularis L. is an annual herb found in throughout India and also many tropical countries. The leaves are tasty and sour. It shows activity of laxative, stimulant, tonic and aphrodisiac. The seeds remove tumors, pain stomach troubles, skin diseases and scabies. It is useful in discharging ulcers5. Powder of entire plant is used as tonic to anemic patient6. Corchorus fascicularis L. shows physiological activity7. Preliminary phytochemical study of leaves of Corchorus fascicularis L. shows that presence of flavonoids, terpenoids, steroids, phenol & tannins, saponins, glycosides and alkaloids8. In Ayurvedic system of medicines this plant has a large demand due to its uses in the treatment of many chronic and acute diseases and disorders. In continuation of work of phytochemical studies of various plants we are presenting of this paper on Corchorus fascicularis L9, 10.

MATERIAL AND METHODS

PLANT MATERIAL COLLECTION AND AUTHENTICATION

The leaves of plant Corchorus fascicularis L. were collected from village Tande of Shirpur tehasil in Dhule district (MS). The specimens of plants were authenticated by Dr. L.K. Kshirsagar, Department of Botany, S.S.V.P.S's L. K. Dr. Ghogrey Science College, Dhule (MS). The dried uniform leaves powder was used for the extraction of constituents of the plant, determination of ash values, extractive values and phytochemical investigation.

DRYING AND PULVERIZATION

Leaves of Corchorus fascicularis L. were shade dried and pulverized and stored in an air tight container for future use.

EXTRACTION OF POWDERED LEAVES

The extraction of Corchorus fascicularis L. leaves were carried out using known standard procedures 11. The powdered leaves were successively extracted by cold maceration process using organic solvents like ethanol.

n-hexane, chloroform and water. All the extracts were evaporated to dryness and stored for future use.

PRELIMINARY PHYTOCHEMICAL SCREENING

The extracts were subjected to preliminary Phytochemical screening for the presence of different chemical groups of compounds. Air dried powdered plant material were screened for the presence of saponins, tannins, flavonoids, steroids, triterpenoids, proteins, glycosides, carbohydrates as described in literatures12,13.

TEST MICROORGANISMS AND GROWTH MEDIA

S. aureus (NCIM 2079), E. coli (NCIM 2169) and fungal strain C. albicans (NCIM 3471) were chosen based upon their clinical and pharmacological importance14. The bacterial strains obtained from NCIM Pune were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 Hrs. at 37 0C on Nutrient Agar and MGYP respectively, following refrigeration storage at 4 0C. The bacterial strains were grown in Muller Hinton agar at 37 0C whereas the yeast were grown in MGYP respectively at 28 0C. The stock cultures were maintained at 4 0C.

ANTIMICROBIAL ACTIVITY15

In vitro antibacterial and antifungal activity were examined for ethanol, n-Hexane, chloroform and water extracts. Antibacterial and antifungal activities of these extracts against two pathogenic bacteria and one pathogenic fungus were investigated by the Agar Disk Diffusion method16, 17, 18. All the extracts were screened for their antibacterial and antifungal activities against the S aureus, E. coli and fungi strain C. albicans. The dilutions of C. fascicularis L. extracts and standard drugs were prepared in double distilled water using nutrient agar tubes. Muller Hinton sterile agar plates were seeded with bacterial strains (1 x 108 bacteria/ ml) and allowed to stay at 37 0C for 3 hrs. Control experiments were carried out under similar condition by using Chloroamphenicol for antibacterial activity and Nyastatin for antifungal activity as standard drugs. All the plates were incubated at 37°C for 18 to 24 hrs for bacteria and at 28°C for 48 to 96 hrs for fungi. The zones of growth inhibition around the disks were measured after 18 to 24 hrs of incubation at 37°C for bacteria and 48 to 96 h for fungi at 28°C, respectively. The sensitivity of the microorganism species to the plant extracts was determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks.

ISOLATION AND PURIFICATION OF COMPOUNDS

A small quantity of ethanol extract was dissolved in ethanol and this solution was spotted on TLC Plates. Silica gel 60F254 precoated plates (Merck) were used for TLC. The spots were detected by spraying 70% Ethanolic-H2SO4 reagent followed by heating. All chemicals and reagents used for TLC were of analytical grade. Then the TLC plates were run by specific solvent system and were viewed individually in iodine chamber and with the 70% Ethanolic -H2SO4 spraying reagent. Through several pilot experiments, it was found that the compounds of chloroform extract fraction were separated by solvent system of Chloroform, Methanol and Ethyl acetate in the proportion of 7:2:1. The ethanol fraction, 10 g, was subjected to column chromatography on silica gel (60-120 mesh) with gradient elution using Chloroform: Methanol: Ethyl acetate19.

Two fractions were found homogeneous on TLC plate by using Chloroform: ethyl acetate (9.2:0.8), Petroleum ether: chloroform (9.5:0.5), Toluene: ethyl acetate:Metahnol (7:2:1) solvent systems. These fractions were crystallized20 and named as EEC-1 (Ethanol extract compound-1) and EEC-2 (Ethanol extract compound-2) respectively.

Test for Flavonoids

Shinoda Test (Magnesium Hydrochloride Reduction Test):

A few crystals of EEC-1 and EEC-2 were dissolved in Ethanol and a magnesium ribbon and drop wise concentrated Hydrochloric acid drop wise added to the solution, for both EEC-1 and EEC-2 formed a crimson red color after few minutes it converted to blue color indicating presence of flavonoids11.

Zinc-Hydrochloride Reduction Test:

A few crystals of EEC-1 and EEC-2 were dissolved in Ethanol; then mixture of Zinc dust and conc. Hydrochloric acid solution was added, both EEC-1 and EEC-2 devloped red color after few minutes indicating presence of flavonoids11.

Alkaline Reagent Test:

A few crystals of EEC-1 and EEC-2 were dissolved in Ethanol. In this solution few drops of Sodium hydroxide solution were added, both EEC-1 and EEC-2 formed an intense yellow color which turns to colorless on addition of few drops of dilute acetic acid indicating the presence of flavonoids.

SPECTROSCOPIC CHARACTERIZATION

Different spectroscopic methods were used to elucidate the structure of EEC-1 and EEC-2. Among the spectroscopic technique IR, 1H-NMR, 13C-NMR and LCMS were carried out. The infrared spectrum was recorded on FTIR 8400 s(Shimadzu), 1HNMR spectra were recorded on a Varian-400 MHZ NMR spectrometer

(Shimadzu), 13CNMR spectra were recorded on a Varian-400 MHZ NMR spectrometer (Shimadzu) at Wockhardt R & D Ltd, Aurangabad, India. The 1HNMR and 13CNMR spectra were recorded using CDC13, as solvent with Tetramethyl silane (TMS) as an internal standard. Mass spectrum was recorded at high resolution on a mass spectrometer (Perkin Elmer Autosystem XL with Turbomass) at Research & Development centre; the data are given in m/z values. Elemental analysis was recorded on Elementar instrument model Vario Micro Cube using oxygen and helium as combustion and carrier gases respectively at a temperature of 1150°C at Wockhardt Research and Development Centre, Aurangabad, India.

RESULT AND DISCUSSION

Physical appearance, color and odor of different extracts were recorded in (Table 1).

Table 1: Shows characteristics of Corchorus fascicularis L. extracts.

Sr. No.	Extract	Physical Appearance	Color	Odor
1	Ethanol	Semi-Solid mass	Dark Green	Pungent Aromatic
2	N- Hexane	Syrupy mass	Light Green	Aromatic
3	Chloroform	Semi-Solid mass	Dark Green	Aromatic
4	Water	Semi-Solid mass	Greenish Brown	Pungent Aromatic

The physical constants evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash value is important in evaluation of purity of drugs i.e. presence or absence of foreign inorganic matter. The ash values, extractive values and moisture content of leaves were determined and results are shown in (Table - 2)

Sr.No.	Parameters	Values (%) w/w
1	Loss on drying	3.02%
2	Ash values:	
	Total ash	5.40%
	Acid insoluble ash	2.96%
	Water soluble ash	1.85%
	sulphated ash	0.64
3	Extractive values:	
	Water soluble extractives	5.54%
	Alcohol soluble extractives	2.90%
	Petroleum ether soluble extractives	1.73%

Phytochemical tests for the presence of secondary phytoconstituents showed following results (Table -3)

Table 3: Show preliminary Phytochemical screening of Corchorus fascicularis leaves powder.

Sr. No.	Phytoconstituents	Ethanol	N-Hexane	Chloroform	Water
1	Alkaloids	–	+	–	–
2	Carbohydrates	+	–	–	–
3	Glycosides	+	–	–	–
4	Flavonoids	+	–	–	–
5	Phenol& Tannins	+	–	–	–
6	Steroids	–	–	–	–
	Terpenoids	+	+		–
8	Saponins	–	–	–	–
9	Proteins	+	–	–	–
10	Amino Acids	+	–	–	–

The anti microbial activity of all the extracts of C. fascicularis L. were studied with concentration 100 ?g/ml against two pathogenic bacterial strains and one fungal strain. Antibacterial and antifungal potential of extracts assessed in terms of zone of inhibition of bacterial growth. The results of antimicrobial activities are presented in Table 1-2. The growth of inhibition zone measured ranged from 15-18 mm for sensitive bacteria and ranged from 08-10 mm for fungal strains. The graphical results are presented in figure 1 and 2.The inhibitory effect of C. fascicularis L. leaves ethanol, n-Hexane, chloroform and aqueous extracts showed at 17.76, 13.61, 15.00, 15.01 mm for E. coli, 16.30, 15.41, 14.18, 15.09 mm for S. aureus and 9.80, 8.15, 9.73, 8.92 for C. albicans respectively, The results showed that C. fascicularis L. leaves extracts were found to be effective against all the microbes tested.

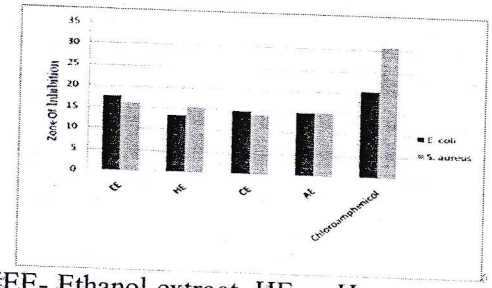
Table 1: Antibacterial activity of C. fascicularis Lam against bacterial test organism.

Microorganism	Zone Of Inhibition in mm Concentration in 100 µg/ml				
	Ethanol Extract	n-Hexane Extract	Chloroform Extract	Aqueous Extract	Chloroamphenicol Standard
E. coli	17.76	13.61	15.00	15.01	20.52
S. aureus	16.30	15.41	14.18	15.09	30.94

Table 2: Antifungal activity of extracts of C. fascicularis L. against bacterial test Organism.

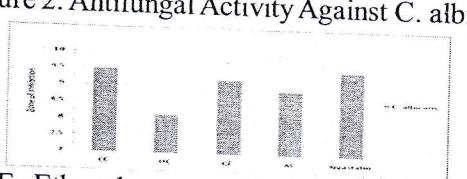
Microorganism	Zone Of Inhibition in mm Concentration in 100 µg/ml				
	Ethanol Extract	n-Hexane Extract	Chloroform Extract	Aqueous Extract	Nyastatin Standard
C. albicans	9.80	8.15	9.73	8.92	9.53

Figure 1: Antibacterial Activity Against E. coli and S. Aureus.



(#EE- Ethanol extract, HE- n-Hexane extract, CE-Chloroform extract, AE- Aqueous extract)

Figure 2: Antifungal Activity Against C. albicans.



(#EE- Ethanol extract, HE- n-Hexane extract, CE-Chloroform extract, AE- Aqueous extract)

From the positive tests for Flavonoids given by the EEC-1 and EEC-2, they were assumed to be flavonoids. The melting point of EEC-1 and EEC-2 were 1400C and

2730C respectively. The UV ?max value of EEC-1 and EEC-2 was formed at 251 and 276 nm, respectively. Mass spectrum of EEC-1 and EEC-2 showed a parent molecular ion peak at 302 and 290 respectively, which corresponds to the molecular formula C15H10O7 (fig.1) and C15H14O6 (fig.2).

In the IR spectrum of EEC-1 and EEC-2, an intensely broad band at 3368 and 3220 cm-1 showed presence of OH stretching and in the 1H-NMR spectrum of EEC-1 it was seen that H-3 proton appeared at ?12.49 as hydroxyl proton with carbonyl system. The signal at 10.74 strong singlet for H-5 due to aromatic hydroxyl group and downfield due to near electronic withdrawing group. In 13C-NMRspectrum, the signal corresponding to the ketonic carbonyl group C-4 appeared at ? 176.51.The signals at ?164.56, ?161.40, ?148.37, ?145.73, ?136.40 for five hydroxyl groups (Table-1).

The 1H-NMR data of EEC-2 showed that the signal at ? 9.14 due to aromatic phenolic groups. The signals at ? 6.70, ? 6.58, ?6.56, ? 5.86, ? 5.66 due to different five aromatic protons. In the 13C-NMR spectra, signals appeared at ? 28.52, 66.98, 81.64 due to C-4, C-3, C-2 carbons respectively and other aromatic carbons showed peaks at ? 94.52, ? 95.79, ? 99.73, ? 115.19, ? 115.75, ? 119.10, ? 131.27, ? 144.01, ? 145.51, ? 156.02, ? 156.84, ? 157.12 (Table-2). From above observation EEC-1 and EEC-2 were found to be Quercetin with reported values21 and Catechin with reported values22.

Table 1: Spectroscopic data of EEC-1 (Quercetin)

Spectroscopic Technique	Data
CHN Analysis	C= 59.56 %, H= 3.518%
UV λ max	256 nm
IR (CHCl3)	3368, 3082, 2840, 1760, 1673,1522,1457, 1430, 1365, 1096, 1014, 716, 691 cm-1
LCMS	302
1HNMR (CDCl3)	δ 12.49 (ss, OH C-5), 10.74 (ss, OH C-7), 9.55 (s, OH C3'), 9.33 (s, OHC-4'), 9.27 (s, OH C-3), 7.66 (d, 1H H-2'), 7.53 (dd, 1H, H-6'),6.87 (d, 1H, H-5'), 6.38 (d, 1H, H-8), 6.16 (d, 1H, H-6)
13CMR (CDCl3)	δ176.5 (C-4), δ 164.56 (C-7), δ 161.40 (C-5), δ 156.81 (C-9), δ 148.37 (C-4'), δ 147.48 (C-2), δ 145.73 (C-3'), δ136.40 (C-3), δ 122.63 (C-1'), δ 120.65 (C-6'), δ 166.28 (C-5'), δ 115.74 (C-2'), δ 103.69 (C-10), δ98.85 (C-6), δ 94.02 (C-8).

Table 2: Spectroscopic data of EEC-2 (Catechin)

Table 2: Spectroscopic data of EEC-2 (Catechin)	
Spectroscopic Technique	Data
CHN Analysis	C=61.13 %, H= 5.080 %,
UV λ max	251 nm
IR (CHCl3)	3240, 2853, 1629, 1522, 1473,1376, 1237, 1080,1030, 732, 674.
LCMS	290
1HNMR (CDCl3)	δ 9.14 (m, Phenolic Protons), δ 6.70 (d, 2 H), δ 6.58 (d, 5' H), (Chemical shift in δppm)δ 6.56 (dd, 6' H), δ 5.86 (d, 6H), δ5.66 (d, 8H), δ4.46 (d, H2), δ 3.79 (ddd, 3H), δ 2.64 (dd, 4 H), δ 2.33 (dd, 4' H).
13CMR (CDCl3)	δ157.12 (C-9), δ156.84 (C-7), δ 156.02 (C-5), δ 145.51 (C-3'), δ 144.01 (C-4'), δ 131.27 (C-1'), δ 119.10 (C-6'), δ115.75 (C-5'), δ 115.19 (C-2'), δ99.73 (C-10), δ 95.79 (C-6), δ 94.52 (C-8), δ 81.64 (C-2), δ 66.98 (C-3), δ 28.52 (C-4).

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