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ISOLATION OF CHEMICAL CONSTITUENTS AND EVALUATION OF ANTIDANDRUFF ACTIVITY OF THE LEAVES OF *URTICA DIOICA* L.

Chhavi Singla[1], Mohammed Ali[2*], Shahnaz Sultana[2,3] and Showkat Rassol Mir[2]

[1]School of Health Sciences, Sushant University, Golf Course Road, Huda, Sushant Lok 2, Sector 55, Gurugram, Haryana 122003, India.

[2]Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi - 110 062, India.

[3]College of Pharmacy, Jazan University, Jazan, Saudi Arabia.

***Corresponding Author: Mohammed Ali**

Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi - 110 062, India.

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ABSTRACT

Urtica dioica L. (Urticaceae) is a perennial herb and its leaves are used to treat anemia, asthma, allergy, arthritis, baldness, bronchitis, chicken pox, colds, dandruff, diabetes, diarrhoea, eczema, flu, gingivitis, excess menstrual flow, nettle rash, piles, rheumatism, sinusitis, sprains, tendonitis, ulcers and wounds. This study was intended to isolate and identify the chemical constituents from the leaves of *Urtica dioica* and to evaluate their antidandruff activity. The leaf powder of *U. dioica* was extracted exhaustively with methanol in a Soxhlet apparatus. The concentrated methanolic extract was dissolved in ethanol (95%) and fractionated by partitioning the solution with petroleum ether, chloroform, ethyl acetate and *n*-butanol. The chloroform fraction exhibited marked antidandruff activity against the growth of *Malassezia furfur*. It was adsorbed on silica gel (60-120 mesh) for the preparation of a slurry. The dried slurry was chromatographed over a silica gel column. The column was eluted with petroleum ether, chloroform and methanol, successively, in order of increasing polarity to isolate 2,6,7-trihydroxynaphthalene (6,7– dihydroxy-β-naphthol, **1**), ellagic acid (**2**) and rutin (quercetin- 3- O- rutinoside) (**3**). Their structures were established on the basis of spectral data analysis and chemical reactions.

Dandruff is a skin disorder caused by a fungus *Malassezia furfur*. The petroleum ether, chloroform, ethyl acetate, *n*-butanol and aqueous fractions of the methanolic extract of the leaves of *U. dioica* against *Malassezia furfur* at concentration of 10 mg/ml each showed zone of inhibition between 6.81 – 22.13 mm. Among all the testing portions the chloroform fraction exhibited marked antidandruff activity. The zone of inhibition in mm was in order of 22.13> 16.47> 12.0> 7.53> 6.81 for chloroform, aqueous, *n*-butanol, ethyl acetate and petroleum ether extracts, respectively.

KEYWORDS: *Urtica dioica* L., leaves, phytoconstituents, isolation, characterization, antidandruff activity.

INTRODUCTION

Urtica dioica L., syn. *U. galeopsifolia* Wierzb. ex Opiz and *U. tibetica* W.T. Wang (Urticaceae), known as chorat, common nettle, stinging nettle and nettle leaf, is originally distributed in cold regions of Europe, temperate Asia, northern Mexico, Pacific Northwest, Canada, the United States and North Africa. The European subspecies has been introduced into Australia, North America and South America. It is a perennial herb, up to 2 m tall; leaves soft, serrated, cordate at the base, opposite, tomentose; flowers tiny, yellow or pink, dioecious; roots tough, yellow; stem erect, creeping, green to purple, square; fruit an achene. The leaves are used to treat anaemia, asthma, allergy, arthritis, baldness, bladder and nasal inflammations, bronchitis, chicken pox, colds, dandruff, diabetes, diarrhoea, eczema, flu, gingivitis, hay fever, head lice, hypertension, insect bites, liver and urinary diseases, excess menstrual flow, nettle rash, piles, benign prostatic hyperplasia, rheumatism, sinusitis, sprains, tendonitis, ulcers and wounds. The whole plant is astringent, depurative, diuretic, galactagogue, haemostatic, hypoglycemic, tonic and a stimulant for hair growth.[1-3] The leaves contained organic acids, glycoprotein, galacturonic acid, carotenoids, flavones, 14-octacosanol, α-dimorphecolic acid, scopoletin, p-hydroxybenzaldehyde, homovanillyl alcohol, phytosterols, aromatic acids, escaletin, tannins, volatile compounds, fatty acids, chlorogenic acid, polysaccharides, isolectins, terpenes, protein, shikimic acid derivatives, caffeoyl malic acid, glucokinnins, vitamins and minerals.[4 -12] The leaf trichomes afforded acetylcholine, histamine, serotonin, leukotrienes and formic acid.[13,14] The roots yielded phenolic

constituents, lignin, 3- and 4-hydroxybenzaldehydes, aromatic alcohols, acetophenones, phytosterols, triterpenoids, polysaccharides, vanillin, lariciresinol derivatives and neo-olivil.[15 – 18] The plant essential oil was composed of carvacrol, carvone, naphthalene, (E)-anethol, hexahydrofarnesyl acetone, (E)-geranyl acetone, (E)-β-ionone, phytol, 2-methyl-2-hepten-2-one, acetophenone, ethyl ketone.[19,20] The flowers possessed flavonol glucosides, scopoletin, caffeoyl malic acid, chlorogenic acid and isorhamnetin-3-O-neohesperidoside.[21]

Excessive shedding of skin cells from the scalp caused a scalp disease known as dandruff. It is faced by all group people and a yeast viz., *Malassezia furfur* [syn. *Pityrosporum ovale*] is responsible for production of this disease.[22] Malassezia fungus transforms the sebum lipid into fatty acids and triglycerides, which accelerate hyperproliferation of keratinocytes.[23] Various pharmaceutical formulations including lotions, ointments and shampoos are applied to manage dandruff. Imidazole derivatives, ketoconazole, salicylic acid, selenium sulphide, tar derivatives and zinc pyrithione are the key ingredients of these formulations.[24] Application of the synthetic formulations was unable to prevent reoccurrence of dandruff and showed various side effects.[25] Utilization of antidandruff herbal preparations is safe. Some essential oils exhibited antifungal effect.[26-28] Plant extracts showed significant activity against dandruff causing organism *Malassezia furfur*. From the results, we conclude that plant extracts have antifungal activity and could be safely used for treating dandruff. Further studies can be made on the active molecules of plant extracts responsible for antidandruff activity.[29] Keeping in view the various therapeutic values of *U. dioica* and the development of ecofriendly, biodegradable and safer herbal preparations its leaves were screened to evaluate antidandruff activity and to isolate and characterize chemical constituents.

MATERIALS AND METHODS

The protocols of all methodologies (procedures, experimental designs and analysis assays) were adopted from the earlier published work.[30,31]

General procedures

Melting points were determined on a Perfit melting point apparatus and are uncorrected. UV spectra were determined on Shimadzu-120 double beam spectrophotometer with methanol as a solvent. IR spectra were recorded in KBr pellet on Shimadzu FTIR-8400 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were scanned on Bruker DRX 300 MHz instrument using TMS as an internal standard and coupling constants (J values) are expressed in Hertz (Hz). Mass spectra were recorded by affecting electron impact ionization at 70 eV on a Jeol SX-102 mass spectrometer equipped with direct inlet prob system. The *m/*z values of the more intense peaks are mentioned and the figures in bracket attached to each *m/*z values indicated relative intensities with respect to the base peak. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G 60 F_{254} precoated TLC plates (Merck, Mumbai, India). Spots were visualised by exposing to iodine vapours, UV radiations (254 and 366 nm) and spraying with ceric sulphate solution.

Plant Material

The leaves of *Urtica dioica* were collected from the Botanical garden of the Forest Research Institute (FRI), Indian Council of Forestry Research Education, P.O. New Forest, Deharadun-248006, and identified by Dr. S. Biswas, Botany Division, FRI. A voucher specimen (MSIP/ 2010/ 08/ 14) was deposited in the herbarium section of the Pharmacognosy Division, Maharaja Surajmal Institute of Pharmacy, Janakpuri, New Delhi.

Extraction

The dried powdered aerial parts of *U. dioica* (2.0 kg) were extracted with methanol exhaustively in a Soxhlet apparatus for 40 hrs. The methanolic extract was concentrated on a steam bath and dried under reduced pressure to get a dark brown viscous mass (301 g). The crude methanolic extract (200 g) was dissolved in ethanol (95%) and fractionated into various fractions by partitioning the solution successively with petroleum ether (60-80ºC), chloroform, ethyl acetate and *n*-butanol to obtain petroleum ether soluble fraction (54 g), chloroform fraction (87 g), ethyl acetate fraction (42 g) and *n*-butanol fraction (25 g).[32]

Total aqueous extract of the leaves of *U. dioica* was prepared by extracting the leaf powder (100 g) with hot water at 80-83°C for 8 hrs. The extract was cooled down at room temperature, filtered through Whatman No. 1 filter paper and the filtrate dried under reduced pressure using rotary evaporator to obtain a dark brown viscous mass (14.1 g).

Antidandruff activity

The cup plate method was used to determined antidandruff activity through a solidified agar layer in a Petri dish or plate. The growth of the added micro-organism was prevented entirely in a zone around the cylindrical hole containing a solution of the antifungal.

Micro-organism

A standard isolate of *Malassezia furfur* was procured from the Institute of Microbial Technology, Chandigarh, India (strain No. MTCC 1374) for the *in vitro* study. The petroleum ether, chloroform, ethyl acetate and *n*-butanol fractions of the methanolic extract of the leaves of *B. dioica* and total leaf aqueous extract were evaluated for most potential extract responsible for antidandruff activity (Bioassay guided). The isolate was maintained in Sabouraud's dextrose agar (SDA) supplemented with corn oil.

Media

Sabouarauds dextrose agar medium was prepared by taking dextrose (20 g), peptone (10 g), agar (20 g) and sterilized corn oil (5 ml) at pH 5.6 and temperature 25 °C. The material was suspended in 1 lit of distilled water. It was heated to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (121 ° C) for 15 minutes.

Preparation of test microorganism

For antidandruff activity evaluation *Malassezia furfur* (MTCC 1374) was maintained on Sabouraud-dextrose agar media and incubated at 32ºC for 96 hours. After completion of incubation, the growth on the slant was washed with 10 ml sterile normal saline with vortexing. Culture concentration was taken at 560 nm by spectrophotometer. A sterile swab was dipped into the slant containing normal saline and excess inoculums were removed by pressing the swab against the inner wall of the test tube. Inoculums were uniformly spread over the plate and left at room temperature for 20 minutes to dry. Wells were punched in each plate with the help of 6 mm steel agar borer and filled with 10 mg/ml extract solution. Plates were incubated at 32 ºC for 96 hours. Results were noted at the end of incubation period.[33, 34]

Zone of inhibition study

Diffusion dependent activities of various extracts were studied by zone of inhibition. The broth culture was uniformly swabbed onto the surface of the Sabouraud's medium. All the extracts were dissolved in their respective solvents at 10 mg/ml concentration. The plates were incubated at 32°C for 96 hours and the zone of inhibition was measured as follows:

1. A 24 hrs broth culture was swabbed over the surface of Sabouraud's agar and all the extracts were dissolved in their respective solvents at 10 mg/ml concentration.
2. A well of 6 mm diameter was cut at the centre of the agar and above prepared different test extracts were loaded on the well.
3. The zone of inhibition of respective solvents were taken as blank and ketoconazole was taken as a standard.
4. The plates were incubated at 32 ° C for 96 hrs.
5. After incubation the zone of inhibition was measured and recorded.

Table 1: Zone of inhibition (mm) of different test extracts of the leaves of *Urtica dioica* and standard ketoconazole against *Malassezia furfur*.

Extract	Concentration	Zone of inhibition (mm) mean± SD
Petroleum ether	10 mg/ml	6.81 ± 0.17
Chloroform	10 mg/ml	22.13 ± 0.35
Ethyl acetate	10 mg/ml	7.53 ± 0.15
n-Butanol	10 mg/ml	12.0 ± 0.26
Total aqueous	10 mg/ml	16.47 ± 0.65
Ketoconazole (standard)	10 µg/ml	25.01 ± 0.03

The above mentioned readings are inclusive of disc diameter.
Values are expressed as mean ± Standard deviation, where n = 3.

Determination of Minimal Inhibitory Concentration (MIC)

Dilution susceptibility testing method was used to determine the minimal concentration of the leaves of *Urtica dioica* extracts to inhibit growth of *Malassezia furfur*. This was achieved by dilution of the extracts to inhibit the micro-organism and was achieved by dilution of extract in either agar or broth media (PDA). Procedure for performing the minimum inhibitory concentration test inoculums preparation was performed as discuss earlier in well diffusion method.

Procedure

1. Different concentrations of plant leaf extracts in (10 µl, 20 µl….up to 100µl) to respective tubes were added.
2. From the inoculums 10 µl of each culture was inoculated separately in each set so that final concentration of fungus in tubes became 106 cells/ml. This procedure was performed for all the positive extracts antifungal activity which were obtained by primary screening.
3. Then all sets of tubes of dilution broth were incubated at 37 °C for 24 hours in an incubator.

All sets of tubes were observed for determination of MIC to the susceptible fungus tested and note down the results.

Isolation of phytoconstituents

The dried chloroform extract (100 g) was dissolved in minimum amount of methanol and and silica gel for column chromatography was then mixed slowly with continuous mixing until the whole chloroform solution of the extract adsorbed on silica gel particles. It was dried in the air, the large lumps were broken by rubbing and finally passed through a sieve (No. 8) to get uniform particle size. The dried slurry was chromatographed over a silica gel column loaded in petroleum ether (b. p. 60 - 80°C). The column was eluted with petroleum ether, petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, *v/v*) and chloroform successively. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized with solvents.

The isolated compounds were recrystallized to get pure compounds.

6,7– Dihydroxy-β-naphthol (1)

Elution of column with chloroform afforded creamy white amorphous mass of compound **1**, yield 102 mg, R_f 0.39 (petroleum ether- chloroform, 1:1), m. p. 51-55 °C; UV λmax (MeOH): 245, 332 nm; IR υ_{max} (KBr): 3432, 3231, 2981, 2830, 1644, 1530, 1449, 1278, 1119, 968, 899, 816 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.43 (1H, s, D_2O exchangeable, OH), 7.39 (1H, s, H-5), 7.01 (1H, d, J = 2.0 Hz, H-1), 6.89 (1H, dd, J = 2.0, 8.4 Hz, H-3), 6.77 (1H, d, J = 8.4 Hz, H-4), 6.34 (1H, s, H-8), 6.10 (2H, brs, D_2O exchangeable, 2 x OH); ^{13}C NMR ($CDCl_3$): δ 144.40 (C-1), 167.92 (C-2), 120.87 (C-3), 115.54 (C-4), 114.88 (C-5), 147.91 (C-6), 145.39 (C-7), 114.23 (C-8), 125.63 (C-9), 126.15 (C-10); TOF MS *m/*z (rel. Int.): 176 $[M]^+$ ($C_{10}H_8O_3$) (2.1).

Ellagic acid (2)

Elution of column with chloroform–methanol (49:1) furnished colourless amorphous mass of **2**, yield 132 mg, R_f 0.71 (chloroform-acetone, 1:1), m. p. 350 - 352 °C; UV λmax (MeOH): 253, 367 nm (log Ɛ 5.2, 1.8); IR υ_{max} (KBr): 3496, 3283, 2927, 2843, 1722, 1667, 1613, 1540, 1426, 1385, 1317, 1265, 1219, 1026, 900, 865 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.01 (2H, brs, 2 x CH= C); TOF MS *m/*z (rel. Int.): 302 $[M]^+$ ($C_{14}H_6O_8$) (14.3).

Rutin (3)

Elution of column with acetone–methanol (4:1) gave creamy amorphous mass of compound **3**, 69 mg, R_f 0.34 (chloroform - methanol, 9:1), m. p. 241-243 °C; UV λmax (MeOH): 266, 358 nm; IR υ_{max} (KBr): 3428, 3365, 3281, 2985, 2841, 1668, 1599, 1505, 1456, 1362, 1295, 1203, 1132, 1092, 1012, 969 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.56 (1H, d, J = 2.1 Hz, H- 2′), 7.52 (1H, dd, J= 2.1, 8.7 Hz, H- 6′), 6.84 (1H, d, J = 8.7 Hz, H-5′), 6.33 (1H, d, J = 2.3 Hz, H - 6), 6.16 (1H, d, J = 2.3 Hz, H - 8), 5.22 (1H, d, J = 7.5 Hz, H-1″), 4.31 (1H, m, H-5″), 3.72 (1H, m, H-2″), 3.33 (1H, m, H-3″), 3.29 (1H, m, H-4″), 3.16 (1H, d, J = 9.6 Hz, H_2-6″a), 3.11 (1H, d, J = 9.6 Hz, H_2 - 6″b), 4.98 (1H, d, J = 4.4 Hz, H-1‴), 4.22 (1H, m, H-5‴), 3.36 (1H, m, H-2‴), 3.31 (1H, m, H-3‴), 3.24 (1H, m, H-4‴), 1.02 (3H, d, J = 6.4 Hz, Me-6‴); ^{13}C NMR (DMSO- d_6): δ 156.86 (C-2), 133.48 (C-3), 177.33 (C-4), 161.18 (C-5), 100.61 (C-6), 164.02 (C-7), 93.48 (C-8), 156.40 (C-9), 103.96 (C-10), 121.12 (C-1′), 116.27 (C-2′), 148.20 (C-3′), 144.42 (C-4′), 121.60 (C-5′), 115.04 (C-6′), 101.99 (C-1″), 73.89 (C-2″), 70.62 (C-3″), 69.64 (C-4″), 76.51 (C-5″), 66.76 (C- 6″), 98.64 (C-1‴), 72.01 (C-2‴), 70.24 (C-3‴), 67.95 (C-4‴), 75.62 (C-5‴), 17.49 (C-6‴); TOF MS *m/*z (rel. int.): 610 $[M]^+$ ($C_{27}H_{30}O_{16}$) (1.2).

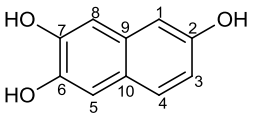
RESULTS AND DISCUSSION

Compound **1** gave positive tests of phenols and showed UV absorption maxima at 245, 332 nm for naphthol and IR absorption bands for hydroxyl groups (3432, 3231 cm^{-1}) and aromatic ring (1644, 1530, 968 cm^{-1}). Its molecular ion peak was determined on the basis of mass and ^{13}C NMR spectra at *m/*z 176 consistent with a molecular formula of a dihydroxy naphthol, $C_{10}H_8O_3$. The ^1H NMR spectrum displayed two one-proton singlets at δ 7.39 and 6.34 assigned to para- coupled aromatic H-5 and H-8 protons, respectively, two one-proton doublets at δ 7.01 (J = 2.0 Hz) and 6.77 (J = 8.4 Hz) ascribed correspondingly to meta-coupled H-1 and ortho-coupled H- 4 and a one-proton double doublet at δ 6.89 (J = 2.0, 8.4 Hz) accounted to meta-, ortho-coupled aromatic H- 3 protons. Two D_2O exchangeable singlets at δ 7.43 (1H) and 6.10 (2H) were due to hydroxyl protons. The ^{13}C NMR spectrum of **1** showed ten carbon signals in the deshielded region between δ 167.92 - 114.22 attributed to the aromatic carbons of naphthol-type molecule. The DEPT spectrum of **1** indicated the presence of five each of methine and quaternary carbons. On the basis of these evidences the structure of **1** has been elucidated as 2,6,7-trihydroxynaphthalene (6,7–dihydroxy-β-naphthol) (F1g. 1).

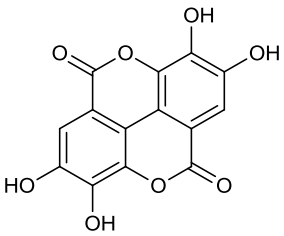
Compound **2**, $[M]^+$ at *m/*z 302 ($C_{14}H_6O_8$), responded positive tests for phenols and displayed UV absorption maxima at 253 and 367 nm for ellagic acid and IR absorption bands for hydroxyl groups (3496, 3283 cm^{-1}), ester functions (1722 cm^{-1}) and aromatic ring (1613, 1540, 1026 cm^{-1}). The ^1HNMR spectrum of **2** exhibited a two-proton singlet at δ 7.01 assigned to aromatic protons. The physical parameters of **2** were identical to ellagic acid. These evidences led to identify **2** as ellagic acid (F1g. 1).[35,36]

Compound **3** gave positive tests for glycosides, had UV absorption maxima at 266 and 358 nm for flavone nature and showed IR absorption bands for hydroxyl groups (3428, 3365, 3281 cm^{-1}), keto group (1668 cm^{-1}) and aromatic ring (1599, 1505, 1012 cm^{-1}). Its molecular ion peak was determined at *m/*z 610 on the basis of mass and ^{13}C NMR spectra corresponding to a molecular formula of a flavone diglycoside, $C_{27}H_{30}O_{16}$. The ^1H NMR spectrum of **3** exhibited four one-proton doublets at δ 7.56 (J = 2.1 Hz), 6.84 (J = 8.7 Hz), 6.33 (J = 2.3 Hz) and 6.16 (J = 2.3 Hz) and a one-proton double doublet at δ 7.52 (J = 2.1, 8.7 Hz) assigned to flavone H-2′, H-5′, H-6, H -8 and H-6′ protons, respectively. Two one-proton doublets at δ 5.22 (J = 7.5 Hz) and 4.98 (J = 4.4 Hz) were ascribed to anomeric H-1″ and H-1‴ protons, respectively. A three-proton doublet at δ 1.02 (J = 6.4 Hz) was due to secondary C-6‴ methyl protons of the rhamnose unit. The other sugar protons resonated from δ 4.31 to 3.11. The ^{13}C NMR spectrum of **3** displayed carbon signals at δ 156.86 (C-2), 133.48 (C-3), and 177.33 (C-4) suggesting a flavone-type molecule. Two carbon signals at δ 101.99 and 98.64 were accounted to the anomeric C-1″ and C-1‴ carbons, respectively. A carbon signal at δ 17.49 was due to C-6‴ methyl carbon indicating rhamnose unit. The other sugar carbons appeared from δ 76.51 to 66.76. The presence of the oxygenated methylene carbon in the deshielded region at δ 66.76 (C-6″) suggested attachment of the

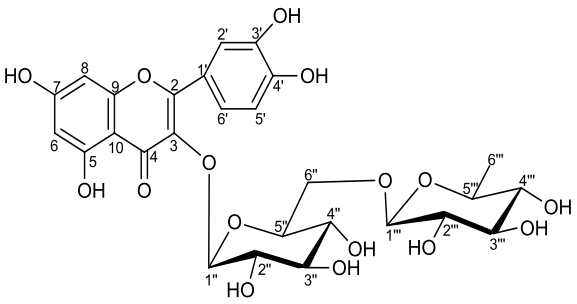
rhamnose sugar at this carbon similar to rutinose. The DEPT spectrum of **3** exhibited the presence of one methylene, one methyl, fifteen methine and ten quaternary carbons in the molecule. The ^{1}H-^{1}H COSY spectrum of **3** showed correlation of H- 6 with H- 8; H- 6′ with H- 2′ and H- 5′; H-1″ with H- 2″, H- 3″ and H- 5″; H_2- 6″ with H- 5″, H- 4″ and H-1‴; and Me-6‴ with H-5‴ and H-4‴. The HSQC spectrum of **3** exhibited interactions of H-6 at δ 6.33 with C-6 at δ 100.61; H-8 at δ 6.16 with C-8 at δ 93.48; H- 2′ at δ 7.56 with C- 2′ at δ 116.27; H-5′ at δ 6.84 with C- 5′ at δ 121.60; H- 6′ at δ7.52 with C- 6″ at δ 115.04; H-1″ at δ 5.22 with C-1″ at δ 101.99; H_2- 6″ at δ 3.16 and 3.11 with C- 6″ at δ 66.76 and Me- 6‴ at δ 1.02 with C- 6‴ at δ 17.49. The ^{1}HNMR and ^{13}C NMR spectra data of **3** were compared with spectral data of rutin. On the basis of these evidences the structure of **3** has been established as quercetin- 3-O-rutinoside (rutin) (F1g. 1).[37-39]



6,7-Dihydroxy-β-naphthol (1)



Ellagic acid (2)



Rutin (3)

Fig. 1: Chemical constituents 1 – 3 isolated from the leaves of *Urtica dioica*.

The zones of inhibition (mm) of different test fractions of the methanolic extract of the leaves of *Urtica dioica* against *Malassezia furfur* are tabulated in Table 1 and compared with ketoconazole taken as a standard. The minimal concentration of plant extracts to inhibit growth of *Malassezia furfur* was determined using dilution susceptibility testing method. This was achieved by dilution of the solvent fractions to inhibit the micro-organism and was achieved by dilution of fractions in either agar or broth media. The petroleum ether, chloroform, ethyl acetate, *n*-butanol and aqueous fractions of the leaves at concentration of 10 mg/ml each showed zone of inhibition between 6.81 – 22.13 mm. The standard ketoconazole at the same concentration exhibited the zone of inhibition of 25.01 mm. The petroleum ether fraction showed minimum antifungal activity (6.81 mm) and the chloroform fraction had nearly the same inhibition effect (22.13 mm) when compared with the control compound. Among all the testing portions the chloroform fraction of the methanolic extract of the leaves of *U. dioica* exhibited marked antidandruff activity, i.e., the chloroform fraction was the most active part displaying the largest zone of inhibition (22.13 mm) against the growth of *Malassezia furfur*. The zone of inhibition in mm was in order of 22.13 > 16.47 > 12.0 > 7.53 > 6.81 for chloroform, aqueous, *n*-butanol, ethyl acetate and petroleum ether extracts, respectively.

In a study relating to antidandruff activity, *Citrus limon* fruit juice and lemon peel powder showed maximum antifungal activity followed by *Emblica officinalis* fruit (Amla), *Acacia concinna* (Shikakai), *Lawsonia inermis* leaves (Henna), *Aloe babadensis* gel (Aloe vera), *Trigonella foenum graecum* seeds (Fenugreek) and *Sapindus mukorossi* nuts (Reetha). The combinations of lemon juice and henna extract and lemon and amla exhibited the best activity as compared to other combinations. The plant extracts showed a considerable marked inhibition activity against the micro-organism *Malassezia furfur* in comparison to branded antidandruff shampoos. Therefore, the herbal extracts can be used to treat dandruff without any side effects.[29]

CONCLUSION

Phytochemical investigation of a chloroform fraction of the methanolic extract of the leaves of *U. dioica* resulted in the isolation of 6,7-dihydroxy-β-naphthol (**1**), ellagic acid (**2**) and rutin (**3**). This work has enhanced understanding about the phytoconstituents of the plant. These compounds may be used as chromatographic markers for standardization of the plant leaves.

The petroleum ether, chloroform, ethyl acetate, *n*-butanol and aqueous fractions of the methanolic extract of the leaves of *U. dioica* against *Malassezia furfur* at concentration of 10 mg/ml each showed zone of inhibition between 6.81 – 22.13 mm. Among all the testing portions the chloroform fraction exhibited marked antidandruff activity. The zone of inhibition in mm was in order of 22.13 > 16.47 > 12.0 > 7.53 > 6.81 for chloroform, aqueous, *n*-butanol, ethyl acetate and petroleum ether extracts, respectively.

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