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**Research Article**

# Bio-active phytoconstituents from non-polar extracts of *Diospyros lotus* stems and demonstration of antifungal activity in the extracts

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**Abstract**

The objective of this study is to isolate phytoconstituents from *Diospyros lotus* stems extracts and to evaluate antifungal, lectin and trypsin inhibitory activities of the extracts. The stems were extracted with petroleum ether, diethyl ether and chloroform, respectively, three compounds were isolated and identified as *β*-sitosterol, stigmasterol and protocatechic acid from both petroleum ether and ether extracts. Chloroform extract was subjected to silica gel column chromatography using $CHCl_3$ as eluent and an increasing amount of EtOAc and MeOH gradually resulted in the isolation and identification of kaempferol 8-O-methylether, apigenin, kaempferol, kaempferol 3-O-α-rhamnoside and luteolin 7-O-*β*-glucoside. The petroleum ether, diethyl ether and chloroform extracts of *Diospyros lotus* were tested at the concentration of 0.1 mg/ml for their effect against the fungus *Mycosphaerella arachidicola* and for their lectin and trypsin inhibitory activities. The results showed that the extracts exhibited significant antifungal activity but had no hemagglutinating and trypsin inhibitory activities.

**Keywords**: *Diospyros lotus*, antifungal, lectin and trypsin inhibitory, phytochemicals, sterols, flavonoids.

## INTRODUCTION

Antibiotics are one of our most important weapons in fighting bacterial and fungal infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses, not only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is essential to find newer drugs with lesser resistance. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as, terpenoids, flavonoids, glycosides, etc., which have been found *in vitro* to have antimicrobial properties [1-2]. Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine [3]. *Diospyros lotus* L. is a tree native to Middle East and south Asia especially from China and Japan [4]. The literature on *D. lotus* is far from abundant. In traditional medicine, *D. lotus* fruit was used as a sedative, antitussive, antiseptic, antidiabetic, antitumor, astringent, laxative, nutritive and as a febrifuge [5-7], in addition, *D. lotus* fruits are used to treat diarrhea, dry coughs and hypertension [8]. *D. lotus* fruit extract has antioxidant activity that protects against hemolytic damage in both glucose-6-phosphate-dehydrogenase-deficient human and rat erythrocytes [9]. Previous phytochemical studies of *D. lotus* revealed the presence of some fatty acids and non volatile acids [10], terpenes [11] and naphthoquinones [12] in the fruits. Gallic acid, methyl gallate, ellagic acid, kaempferol, quercetin, myricetin, myricetin 3-O-*β*-glucuronide, and myricetin-3-O-α-rhamnoside were isolated from *D. lotus* fruit extract. *D. lotus* fruits extract and compounds demonstrated the highest inhibitory activity against COR-L23 tumor cells [13]. Ellagic acid demonstrated high anti-proliferative activity against both C32 and A375 tumor cells, and gallic

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acid exerted the most potent cytotoxicity against CaCo-2 tumor cells [13]. The antioxidant, anti-hemolytic and nephroprotective activities of *D. lotus* seed extract have been demonstrated *in vitro* as well as *in vivo* [14]. Since gastric phytobezoars are difficult to treat although rare, *D. lotus* consumption is discouraged for patients with a history of gastrointestinal surgery or poor dental and oral health [15]. Genetic variation among *D. lotus* genotypes in Turkey has been reported by Yildirim et al. [16]. In view of the fact that no information concerning *D. lotus* stem is available, we undertook the present study to isolate phytochemicals from *D. lotus* stem. As a part of our screening program to investigate antifungal, lectin and trypsin inhibitory activities from plants, another objective of the present investigation was to assay antifungal lectin and trypsin inhibitory activities from non-polar extracts of *D. lotus* stems.

## MATERIALS AND METHODS

**Experimental:** A Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC, Egypt) was used for spectrophotometric measurements. Spectroscopic data were collected using NMR–Varian MS (Finnigan MAT SSQ 7000, 70 ev). Silica gel (60-200 mesh, Merck) for column chromatography, pre-coated sheets of silica gel 60 $F_{254}$ (Merck) were used for thin layer chromatography (TLC). Sephadex LH-20 (Sigma) was used for gel chromatography.

**Plant material:** The stems of *Diospyros lotus* L. were collected from the Agricultural Research Centre, Giza, Egypt in April 2010 during the flowering season and identified by Dr. Mohammed El-Gebaly, Department of Botany, and National Research Centre (NRC) and by Mrs. Tereez Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen was deposited in the herbarium of Agricultural Research Centre, Giza, Egypt.

**Preparation of plant extracts**: The stems of *D. lotus* (700 g) were extracted with petroleum ether, diethyl ether and chloroform several times until exhaustion. Each extract was concentrated under reduced pressure to give 12.0 g, 9.0 g and 7.5 g respectively. The extract was phytochemically screened using different chemical assays to identify the presence or absence of the phytochemical components in the plant. The method described by Connolly et al. [17] was used for sterols and/or triterpenes; that described by Wolf et al. [18] for carbohydrates and saponins; Harbone [19] for flavonoids and alkaloids; that reported by Farnsworth [20] for coumarins; and that described by Geissman [21] for tannins. Thin layer chromatography (TLC) of both petroleum ether and ether extracts in the solvent system n-hexane:MeOH (95:5, v/v) showed the same profile and so the two extracts were collected and then the total extract was subjected to silica gel column chromatography using n-hexane as eluent and an increasing amount of MeOH gradually. Three compounds were isolated. Compound 1 (β-sitosterol) was isolated by elution with n-hexane:MeOH (95:5). Compound 2 (stigmasterol) was isolated through elution with n-hexane:MeOH (90:10). Compound 3 (Protocatechic acid) was isolated by elution with n-hexane:MeOH (85:15). Chloroform extract was subjected to silica gel column chromatography using $CHCl_3$ as eluent and an increasing amount of EtOAc and MeOH gradually. Compound 4 (apigenin) was isolated by elution with $CHCl_3$:EtOAc (50:50). Compound 5 (kaempferol 8-O-methyl ether) was isolated by elution with $CHCl_3$:EtOAc (70: 30), compound 6 (kempferol) was isolated by elution with EtOAc, compound 7 (kaempferol 3-O-α-rhamnoside) and compound 8 (luteolin 7-O-β-glucoside) were isolated by further eluction with EtOAc and MeOH gradually.

**Acid hydrolysis of flavonoid glycosides:** Solutions of 5 mg of compounds 7, 8 in 5 ml 10 % HCl were heated for 5 h. The reaction mixture was extracted with ethyl acetate. The ethyl acetate fraction (aglycone) and the aqueous fraction (sugars) were concentrated for identification. The sugars were identified by TLC (acetonitrile:water, 85:15) by comparison with authentic samples.

**Assay of antifungal, hemagglutinating, and trypsin inhibitory activities:** The petroleum ether, diethyl ether and chloroform extracts were extracted with phosphate buffered saline or dimethyl sulfoxide until the extracts could no longer go into solution. Then a 50 µl aliquot of each of the phosphate buffered saline-extracted and dimethyl sulfoxide-extracted samples was tested for antifungal activity, and a 50-ul aliquot of each of the phosphate buffered saline-extracted samples was tested for lectin and trypsin inhibitory activities. The assay methods are as follows.

**Assay of antifungal activity:** The assay for antifungal activity against *Mycosphaerella arachidicola* was performed using 100 mm x 15 mm Petri plates containing 10 ml of potato dextrose agar. After the mycelial colony had developed, sterile blank paper disks (0.625 cm in diameter) were placed around and at a distance of 1 cm away from the rim of the mycelial colony. An

aliquot (50 µl) of the sample in 20 mM phosphate-buffered saline (pH 7.2) was introduced to a disk. Commercial available nystatin obtained from Sigma (10 µg) and phosphate-buffered saline were included in the assay respectively as positive and negative control. The plates were incubated at 23 $^{o}$C for 72 h until mycelial growth had enveloped peripheral disks containing the control (buffer) and had produced crescents of inhibition around disks containing samples with antifungal activity.

**Assay of hemagglutinating activity:** The hemagglutinating activity of the lectin was determined by measuring agglutination of rabbit red blood cells in a serial 2-fold dilution of the lectin. A 50 µl of lectin sample and its 2-fold serial dilution in phosphate buffer saline (PBS), pH 7.2 were mixed with 50 µl of 2 % red blood cells suspension in round-bottom 96-well microtiter plates. PBS and commercial available concanavalin A from Sigma were used as negative and positive controls, respectively. Hemagglutination titer was defined as the reciprocal of the highest dilution giving visible agglutination of the rabbit red blood cells after incubation at room temperature for 1 hour.

**Assay of trypsin inhibitory activity:** In the assay of trypsin inhibitory activity, the test sample (20 µl) as added to a solution containing 470 µl 1 % casein solution in 0.1 M Tris–HCl buffer (pH 7.6). Tris–HCl buffer and commercial available soybean trypsin inhibitor from Sigma were used as negative and positive controls, respectively. Then, 10 µl of a 5 mg/ml trypsin solution was added, followed by incubation at 37 °C for 20 min. The reaction was terminated by addition of 500 µl 5 % trichloroacetic acid solution. After centrifugation at 20,000 × *g* for 10 min, the supernatant was collected and OD 280 nm was measured. The activity was calculated by:

Trypsin inhibitory activity (U) = (OD 280nm of control – OD 280nm of sample)/[OD 280nm of control × trypsin (mg)] One unit of trypsin inhibitor activity is defined as the activity capable to inhibit 1 mg trypsin.

## RESULTS AND DISCUSSION

**Results for antifungal, hemagglutinating, and trypsin inhibitory activities:** The extracts exhibited antifungal activity and the results are shown in (Fig. 1a and b) but were not active for hemagglutinating, and trypsin inhibitory activities.

**Results of phytochemical study:** Phytochemical analysis of the fractions of *D. lotus* is included in table 1. Further phytochemical analysis and chromatographic separation and purification of petroleum ether, ether, chloroform resulted in the isolation of stigmasterol, β-sitosterol, and protocatechic acid from petroleum ether and ether extracts which showed the same thin layer chromatography (TLC) profile, while apigenin, kaempferol 8-O-methyl ether, kempferol and kaempferol 3-O-α-rhamnoside and luteolin 7-O-*β*-glucoside were isolated from chloroform extract (Fig. 2) and their structures were elucidated on the basis of UV, $^{1}$H-NMR, $^{13}$C-NMR and MS analyses.

**Structural elucidation of the compounds:**

*β*-sitosterol (1): 20 mg, white needles, $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 5.37 (IH, m, H-6), 3.52 (IH, m, H-3) , 1.09 (3H, s, CH3-19), 0.98 (3H, d, J= 6.5, CH3-21), 0.92 (3H, t, J= 7.4, CH3-29), 0.85 (3H, d, J= 6.7Hz, CH3-26), 0.81 (3H, d, J= 6.7Hz, CH3-27), 0.75 (3H, s, CH3-18). $^{13}$C-NMR(100 MHz, $CDCl_3$): δ 140.4 (C-5), 121.5 (C-6), 71.6 (C-3), 57.2 (C-17), 56.4 (C-14), 50.3 (C-9), 46.3 (C-24), 42,8 (C-13, 4), 39.8 (C-12), 37.6 (C-1), 36.7 (C-10), 35.9 (C-20), 34.2 (C-22), 31.7 (C-8, 7), 31.4 (C-2), 29.2 (C-25), 28.4 (C-16), 26.2 (C-23), 24.5 (C-15), 23.4 (C-28), 21.1 (C-11), 19.8 (C-26), 19.5 (C-19), 19.2 (C-27), 18.6 (C-21).

Stigmasterol (2): 17 mg, white needle crystals, $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 5.32 (IH, m, H-6), 5.11 (1H,dd, J= 14.2, 8.2 Hz, H-22), 5.04 (1H,dd, J= 14.2, 8.2 Hz, H-23), 3.54 (IH, m, H-3), 1.04 (3H, s, CH3-10), 0.9 (3H, d, *J*= 6.5, CH3-20), 0.84 (3H, d, J= 7.4, CH3-27), 0.82 (3H, d, *J*= 7.4, CH3-26), 0.68 (3H, s, CH3-13). $^{13}$C-NMR(100 MHz, $CDCl_3$): δ 140.6 (C-5), 138.4 (C-22), 129.1 (C-23), 121.8 (C-6), 71.9 (C-3), 56.7 (C-17), 56.9 (C-14), 50.9 (C-9), 50.7 (C-24), 42,6 (C-13, 4), 39.6 (C-12), 37.4 (C-1), 40.2 (C-20), 36.7 (C-10), 31.4 (C-8, 7), 31.7 (C-2), 30.9 (C-25), 28.8 (C-16), 24.8 (C-15), 24.7 (C-28), 21.5 (C-11), 20.8 (C-26), 20.4 (C-19), 19.7 (C-27), 19.1 (C-21).

Protocatechic acid (3): 10 mg, yellow powder, λmax (nm), MeOH: 265, 298, (NaOMe): 278, 314. $^{1}$H-NMR (DMSO-$d_6$, 300 MHz): δ (ppm) 7.32 (1H, d, *J* = 2.4 Hz, H-2), 7.25 (1H, dd, *J* = 8 and 2.4 Hz, H-6), 6.78 (1H, d, *J* = 8 Hz, H-5).

Kaempferol 8-O-methylether (4): 11 mg, yellow amorphous powder, λmax (nm), MeOH: 268, 320, 360, (NaOMe) 283, 337, 417. ($AlCl_3$) 244, 313, 355, 438 (AlCl3/ HCl) 244, 313, 354. (NaOAc) 284, 294, 385 (NaOAc/ $H_3BO_3$) 268, 360. $^{1}$HNMR (300 MHz, DMSO-d6): δ ppm 8.0 (2H, d, J = 8.4 Hz, H-2`/6`), 6.88 (2H, d, J = 8.4 Hz, H-3`/5`), 6.28 (1H, s, H-6), 3.72 (1H, s, $OCH_3$).

Apigenin (5): 20 mg, yellow powder, $^{1}$H-NMR: δ 12.8 (s, 1H, 5-OH), 7.6 (d, *J* = 8 Hz, 2H, H-2´,6´),

6.8 (d, *J* = 8 Hz, 2H, H-3´,5´), 6.15 (s, 1H, H-3), 5.83 (d, *J* = 2 Hz, 1H, H-8), 5.42 (d, *J* = 2 Hz, 1H, H-6). (-) ESI-MS: *m/z* 269 $[M-H]^-$.

Kaempferol (6): 12 mg, yellow powder, 1H-NMR (DMSO-d6, 400 MHz): δ 8.11 (2H, d, J = 8 Hz, H-2', 6'), 6.96 (2H, d, J = 8 Hz, H-3',5'), 6.47 (1H, d, J = 2 Hz, H-8), 6.19 (1H, d, J= 2 Hz, H-6). (+) ESI-MS: m/z 287[M+H]+.

Kaempferol 3-O-α-rhamnoside (7): 18 mg, yellow powder. $^1$H-NMR ($CD_3OD$, 400 MHz): δ 7.75 (2H,d, *J*=8 Hz, H-2',6,). 6.9 (2H,d, *J*=8 Hz, H-3',5'). 6.4 (1H, d, *J*= 2 Hz, H-8), 6.2 (1H, d, *J*= 2 Hz, H-6). 5.38 (1H, d, *J*=2 Hz, H-1"), 0.9 ($CH_3$, d, *J* =6 Hz). $^{13}$C-NMR ($CD_3OD$, 100 MHz): δ ppm 179.85 (C-4), 166.2 (C-7), 161.8 (C-5), 159.5 (C-4'), 158.2 (C-2), 136.4 (C-9), 132.2 (C-3),122.9 (C-6'), 116.8 (C-2'), 116.2 (C-3') , 106.1 (C-1'), 103.7 (C-5'), 104.7 (C-10) , 100.1 (C-1"), 95.1 (C-8), 94.9 (C-6), 73 (C-5"), 72.4 (C-3"), 72.3 (C-2"), 72.2 for (C-4"), 17.9 (CH3-rhamnosyl).

Luteolin 7-O-β-glucoside (8): 15 mg, yellow amorphous powder. $^1$H-NMR (DMSO-$d_6$, 400 MHz) : δ 12.8 (s, 1H, 5-OH), 7.5(d, *J* = 8 Hz, 1H, H-6´), 7.48(d, *J*=1.2 Hz, 1H, H-2´), 6.85 (d, J = 8 Hz, 1H, H-5´), 6.72 (s, 1H, H-3), 6.4 (d, 3J = 2 Hz,1H, H-8), 6.15 (d, J = 2.2 Hz, 1H, H-6), 5.0 (d, 2J = 7.5 Hz, 1H, H-1´´).$^{13}$C-NMR (DMSO-$d_6$, 100 MHz): δ 182 (C-4), 164.4 (C-2), 162.6 (C-7), 161 (C-5), 158.7 (C-9), 149.8 (C-4´), 145.7 (C-3´),121.5 (C-1´), 119.3 (C-6´), 116.2 (C-5´), 113.4 (C-2´), 105 (C-10), 103 (C-3), 100 (C-1´´), 99.8 (C-6), 94.5 (C-8), 77.2 (C-5´´), 76.2 (C-3´´), 73.5 (C-2´´), 69.6 (C-4´´), 61 (C-6´´).

Plant extracts are of the most attractive sources of new drugs and have been shown to produce promising results in different pharmacological activities. In the present investigation, phytochemical analysis of the fractions from nonpolar *D. lotus* extracts disclosed the presence of triterpenes and/or sterols in petroleum ether and ether extracts, while flavonoids and triterpenes were detected in the $CHCl_3$ extract. Chromatographic separation and purification of petroleum ether and ether extracts of *D. lotus* resulted in isolation and identification of compound 1 (β-sitosterol) which gave a dark spot under short UV light and changed to violet upon spraying with vanillin-sulphuric acid and heating in an oven at 110 °C for 5 min. NMR spectral data showed signals very close to β-sitosterol [22]. Compound 2 (stigmasterol) which gave a dark spot under short UV light changed to violet on spraying with vanillin sulphuric and heating in an oven at 110 °C for 5 min and comparison with published data in the literature allowed us to identify that compound 2 is stigmasterol [22-23] and confirmation was done by co-TLC with authentic samples. Protocatechic acid (compound 3) gave a deep blue colour under UV light. UV spectra of compound 3 in MeOH shows one main band at 265 by addition of NaOMe give shift of about 13 nm indicating free OH at position 4 of the ring characteristic for a hydroxy phenolic acid [24], $^1$H-NMR indicates presence of three aromatic protons and confirmation was achieved by co-TLC with authentic sample.

Chromatographic separation and purification of $CHCl_3$ extract resulted in isolation and identification of apigenin (compound 4) which gave deep purple colour under UV light changing into a yellow-green colour when exposed to ammonia vapour. It also gave yellow fluorosence colour after spraying with $AlCl_3$ under UV light. $^1$H-NMR and MS spectral data are in agreement with apigenin compound [25], compound 5 (kaempferol 8-O-methyl ether) which gave off orange spot under UV light changed to yellow fluorescence by ($NH_3$) and ($AlCl_3$) under UV light. $^1$H-NMR and 13C-NMR spectral data are in agreement with data of kaempferol 8-O-methyl ether, compound 6 (kaempferol) which gave a yellow colour under UV light and under UV and on exposure to ammonia or spraying with $AlCl_3$ reagent respectively, it gave florescent yellowish green colour. $^1$H-NMR and MS spectral data are in agreement with kaempferol [26]. Compound 7 (kaempferol 3-O-α-rhamnoside) was isolated as a deep purple spot under UV light and on exposure to ammonia or spraying with $AlCl_3$ reagent respectively, it gave a florescent yellow colour, acidic hydrolysis of compound 7 gave kaempferol aglycone and rhamnose sugar, and NMR spectral data are in accordance with those of kaempferol 3-O-α-rhamnoside [27], and compound 8 (luteolin 7-O-β-glucoside) was isolated as deep purple spot under UV light and on exposure to ammonia or spraying with $AlCl_3$ reagent respectively, it gave florescent yellow colour, acidic hydrolysis of compound 8 gave luteolin aglycone and glucose sugar, as well NMR spectral data are in accordance with those of luteolin 7-O-*β*-glucoside [26].

Previously eight compounds had been isolated from *D. lotus* and identified as kaempferol, ellagic acid, gallic acid, methylgallate, myricetin, myricetin 3-O-beta-glucuronide, myricetin-3-O-alpha-rhamnoside, and quercetin. *D. lotus* extract manifested activity in various *in vitro* assays of antioxidant activity (DPPH, ABTS, FRAP, and $Fe^{2+}$ chelating activity assay). *D. lotus* extract demonstrated antiproliferative activity against

COR-L23 with an $IC_{50}$ value of 12.2 μg/ml, ellagic acid displayed antiproliferative activity against C32 and A375 cells with $IC_{50}$ values of 0.8 and 4.1 μg/ml, respectively. Gallic acid showed cytotoxic activity against CaCo-2 ($IC_{50}$ 2.6 μg/ml).The antioxidant activity and antiproliferative activities of *D. lotus* are related to identified phenolic compounds [13]. Kaempferol-3-O-(2"-O-galloyl)-glucoside from *Diospyros kaki* leaves inhibited the activity of angiotensin-converting enzyme [28]. Kaempferol 3-O-beta-D-galactopyranoside and D-glucopyranoside were isolated from *Diospyros kaki* leaves [29]. Kaempferol-3-O-(2"-O-galloyl-β-D-glucopyranoside) (KOG) were isolated from the leaves of persimmon [30]. Fresh persimmon leaves contained regiospecific 2″-galloylated galactosides and glucosides of kaempferol [31].

Kaempferol 3-O-α-L-rhamnopyranosyl-(1→ 2)-β-D-glucopyranoside were isolated from *Diospyros crassiflora* leaves [32]. Kaempferol were isolated from *D. lotus* [13]. Beta-sitosterol, stigmasterol, and stigmast-4-en-3-one were isolated from the n-hexane extract of the stems of *Diospyros maritima* Blume [33]. Stigmasterol and stigmasterol 3-O-β-D-glucopyranoside were isolated from the leaves of *Diospyros crassiflora* (Hiern) [32]. Protocatechuic acid methyl ester was isolated from the methanol extract of *Diospyros melanoxylon* leaves [34]. Thus, with the exception of kaempferol, the present report represents the first isolation and identification of *β*-sitosterol, stigmasterol, protocatechic acid, apigenin, kaempferol 8-O-methyl ether, kaempferol 3-O-α-rhamnoside, and luteolin 7-O-β-glucoside from *D. lotus* extract.

The pharmacological actions of the compounds isolated in the present study have been reported. Kaempferol (3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a flavonoid found in many edible plants (e.g. beans, broccoli, cabbage, endive, grapes, kale, leek, tea, tomato, and strawberries) and in plants or phytomedicinal products (e.g. *Equisetum spp, Ginkgo biloba, Moringa oleifera, Sophora japonica, Tilia spp,* and propolis). Kaempferol and some of its glycosides display a diversity of actions, including analgesic, antiallergic, anticancer, antidiabetic, anti-inflammatory, antimicrobial, antioxidant, antiosteoporotic, anxiolytic, cardioprotective, estrogenic/antiestrogenic, and neuroprotective activities [35].

Protocatechic acid possesses anticarcinogenic, antihyperglycemic, anti-inflammatory, antioxidant, and neuroprotective activities [36]. The biological activities of phytosterols (anti-inflammatory, cholesterol-lowering, anti-microbial, anti-bacterial, anti-fungal, anti-tumor and chemopreventive effects) including β-sitosterol have been summarized [37]. The biological properties of stimasterol have been reviewed by Tlili et al. [38]. Luteolin exhibits an array of pharmacological activities, encompassing anti-allergic, antiangiogenic, anticancer, antioxidant, anti-inflammatory, antimicrobial and activities. Suppression of activities of topoisomerases I and II, NF-kappa B, AP-1, HER2, IGF1R, PI3K, and STAT3, stabilization of p53, and modulation of reactive oxygen species levels are mechanisms implicated in the pharmacological actions of luteolin [39-40].

The chemical identity of the antifungal principle in nonpolar extracts of *Diospyros lotus* stems awaits elucidation but is likely similar to plumbagin [41-42] and 7-methyljuglone isodiospyrin [43], but different from chitinase [44] and thaumatin-like antifungal protein [45] mentioned below. A variety of molecules with antifungal activity have been isolated from other *Diospyros* species. The methanol/dichloromethane extract and plumbagin isolated from the stem bark extract of *Diospyros crassiflora* exerted antifungal activity against yeast pathogens and filamentous fungi: *Aspergillus niger, A. flavus, Alternaria sp., Candida albicans, C. glabrata, C. krusei, C. tropicalis, Cladosporium sp., Cryptococcus neoformans, Fusarium sp., Geotrichum candidum,* and *Penicillium sp.* [41]. 7-methyljuglone isodiospyrin from the acetone extract of *Diospyros virginian* roots exhibited moderate antifungal activity against *P. viticola* and potent antifungal activity against *P. obscurans* [43]. Plumbagin (5-hydroxy-2-methyl-1, 4-naphtoquinone) from the stem bark of *Diospyros canaliculata* demonstrated antifungal activity [42]. The 29-kDa persimmon (*Diospyros kaki*) fruit chitinase inhibited the pathogenic fungus *Trichoderma viride* [44]. A 27-kDa thaumatin-like antifungal protein from the overripe fruits of *Diospyros texana* suppressed the growth of the agronomically important pathogen *Phytophthora infestans*, the causative agent of potato late blight [45]. Protease inhibiting and hemagglutinating (lectin) activities are absent from *D. lotus* extract. These two activities have not been reported from other *Diospyros* species. *Diospyros lotus* fruit extract expressed antioxidant activity which protected both G6PD-deficient human and rat erythrocytes *in vitro* and *in vivo* against hemolytic damage brought about by *Vicia faba* bean extract [9]. The antioxidant activity of *Diospyros lotus* seed extract has been observed in a variety of *in vitro* models i.e., DPPH, nitric oxide and hydrogen peroxide radicals scavenging activity, iron ion

chelating, reducing power and lipid peroxidation through linoleic acid. Antihemolytic activity was demonstrated by protection against hydrogen peroxide-induced erythrocyte hemolysis. Nephroprotective activity was manifested by protection against gentamicin-induced renal injury as evidenced by mitigation of the gentamicin-induced elevation in serum level of blood urea nitrogen and creatinine [14].

## CONCLUSION

The findings of the present investigation are viewed together with observations recorded in the literature, it appears that *D. lotus* stem contains a repertoire of phytochemicals beneficial to health.

Table 1: Phytochemical analysis of *D. lotus* stems fractions

| Chemical Constituents | Pet. Ether extract | Ether extract | Chloroform extract |
|---|---|---|---|
| Carbohydrates and/or glycosides | - | - | - |
| Tannins | | | |
| a. Condensed tannins | - | - | - |
| b. Hydrolysable tannins | - | - | - |
| Alkaloids and/or nitrogenous bases | - | - | - |
| Flavonoids | - | - | + |
| Sterols and/or triterpenes | + | - | + |
| Saponins | - | - | - |
| Coumarins | - | - | - |

\+ denotes the presence of the constituents

\- denotes the absence of the constituents

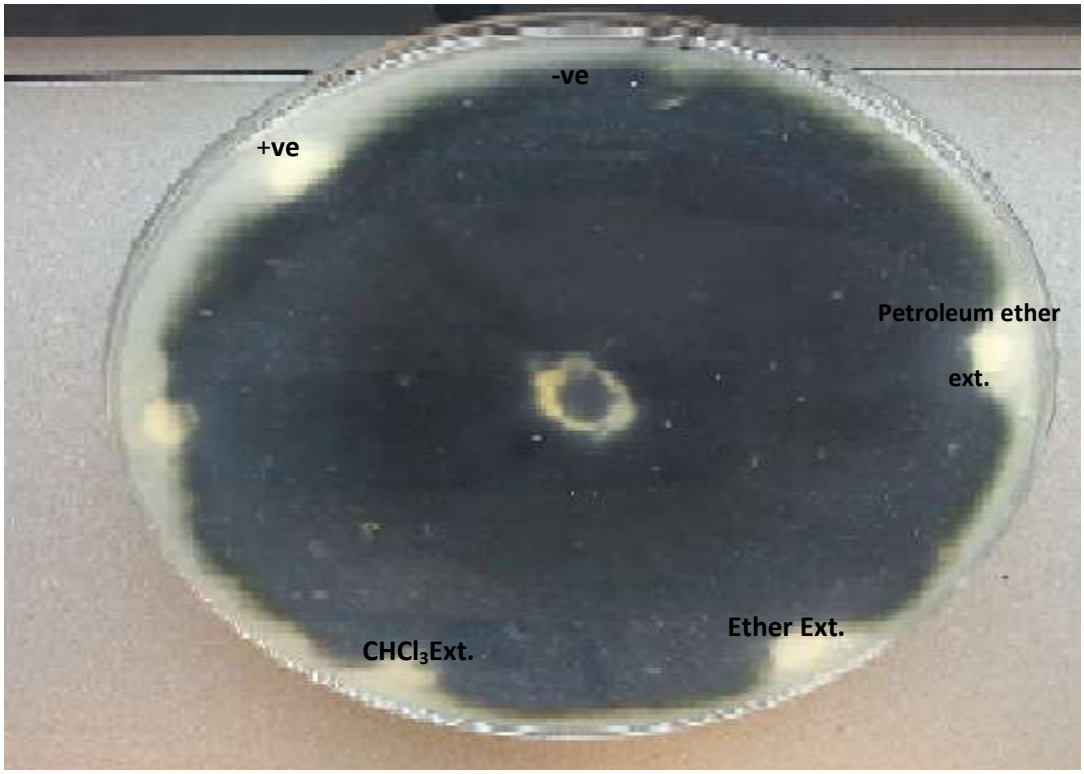



**Figure 1a:** Antifungal activity of different samples toward *M. Arachidicola*. Petroleum ether, diethyl ether and chloroform extracts, were dissolved in PBS. The invagination of the rim of the mycelial colony indicated antifungal activity.

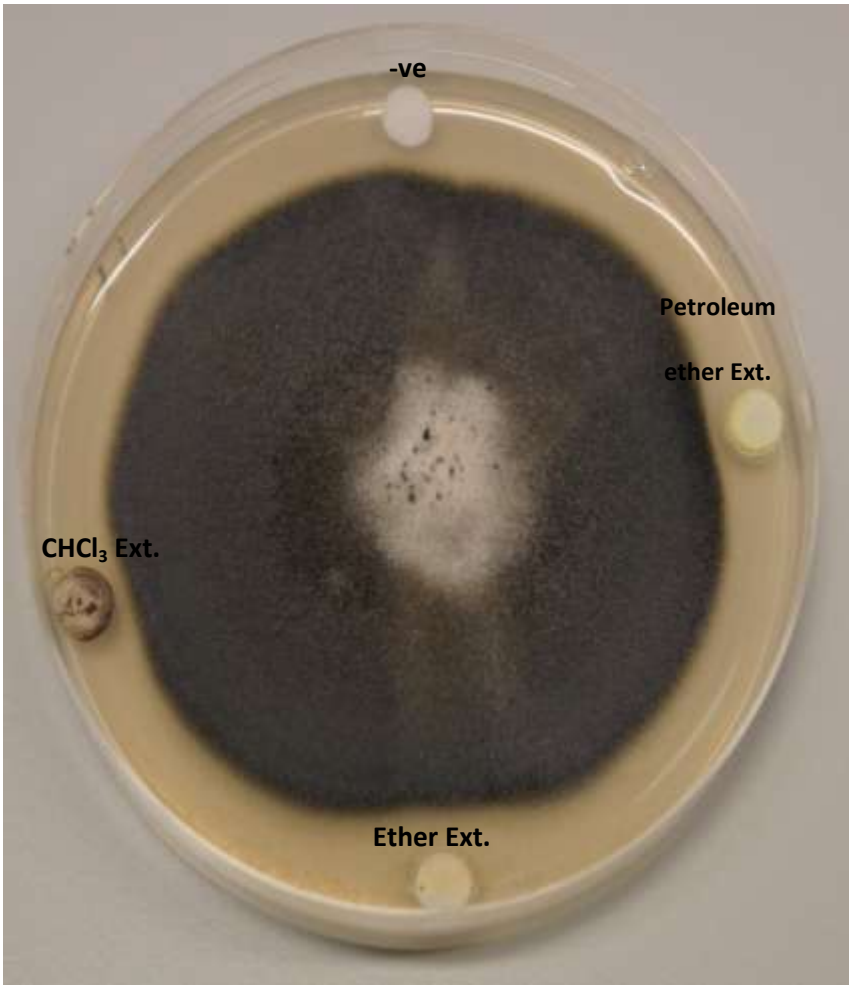
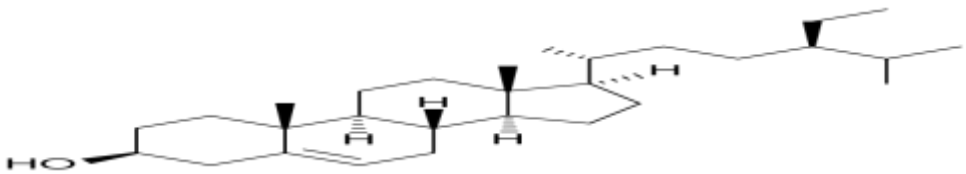
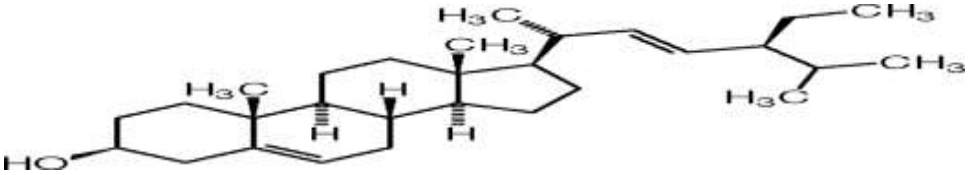


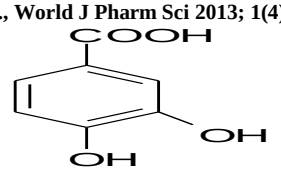

**Figure 1b:** Antifungal activity of different samples toward *M. arachidicola*. The extracts were dissolved in 10 % DMSO, and the concentrations of petroleum ether, diethyl ether and chloroform extracts, were 2.5 mg/ml, 5.0 mg/ml and 4.0 mg/ml respectively. The invagination of the rim of the mycelial colony indicated antifungal activity.



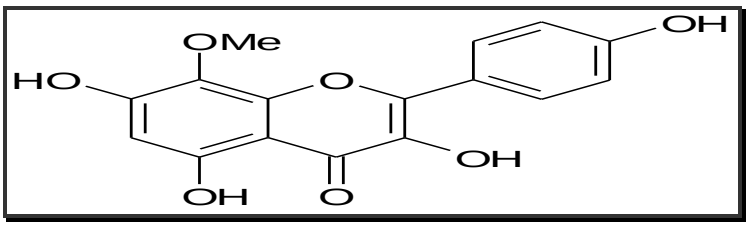
1: *β*-sitosterol



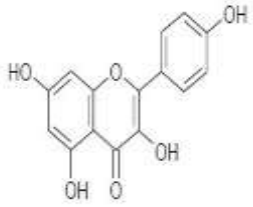
2: Stigmasterol

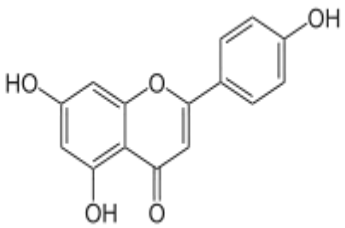


3: Protocatechuic acid

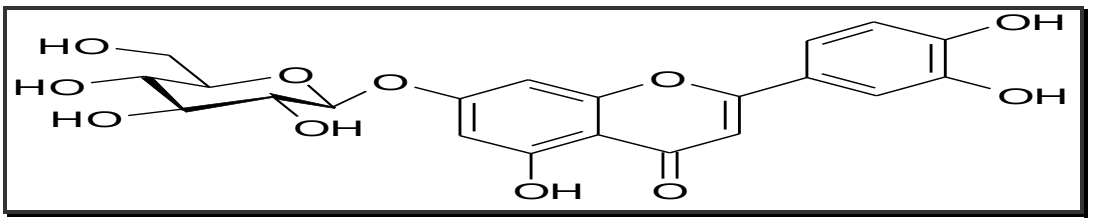


4: Kaempferol 8-O-methyl ether

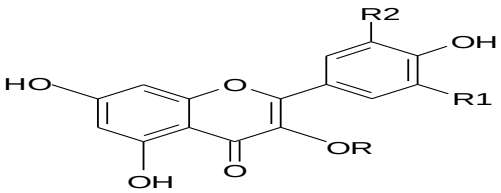




5: Apigenin 6: Kaempferol



7: luteolin 7-*O*-*β*-glucopyranoside



R1=R2=H, R=Rhamnose

8: Kampferol 3-O-α-rhamnoside

**Figure 2.** Compounds isolated from non-polar extracts of *D. lotus* stems

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