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FLAVONOIDS OF *BOERHAVIA DIFFUSA* - GC-MS ANALYSIS AND INHIBITORY ACTIVITY AGAINST PATHOGENIC MICROBES

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Keywords:

Boerhavia diffusa, Bioactive compounds, Kaempferol, Luteolin, Quercetin, GC-MS, Antimicrobial activity

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ABSTRACT: *Boerhavia diffusa* is a species of flowering plant in the four o'clock family, *Boerhavia* belongs to family Nyctaginaceae the plant holds the tremendous potential of medicinal value and has been traditionally used in various ailments like syphilis, leukoderma, blood disorders to name a few. The present study focuses on the GC-MS analysis of extracts of all the plant parts of *B. diffusa* which revealed the presence of certain bioactive compounds like kaempferol, luteolin, quercetin and so forth. A total of about 20 bioactive compounds were identified. In *Boerhavia diffusa* in all plant parts roots, stems and leaves kaempferol is observed maximum. Total amount of flavonoids was maximum in leaves and minimum in stem. Antimicrobial activity of the extracts was assayed against pathogenic bacteria and fungi. In *B. diffusa*, flavonoids extracted from leaf were highly active against *F. oxysporium*, against bacterial strains flavonoids from leaves were highly active and showed maximum activity against *E. coli*. The study thus infers that the presence of bioactive components may be the principle behind the antimicrobial property of different plant parts and therefore *Boerhavia diffusa* forms a potential plant for herbal drug formulation.

INTRODUCTION: Healing with medicinal plants is as old as mankind itself. Herbal plants, the oldest form of healthcare known to mankind. Plants are a goldmine of novel chemicals; much impressive number of modern drugs has been developed from them [1, 2]. *Boerhavia diffusa* is a species of flowering plant in the four o'clock family which is commonly known as tar vine, punarnava meaning that which rejuvenates or renews the body, or red spiderling. It is widely dispersed and is found in the tropical, subtropical and temperate regions of the world.

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Despite this, the Nyctaginaceae have attracted little attention from botanists [3, 4]. Flavonoids or bioflavonoids (from the Latin word flavus meaning yellow, their color in nature) are a class of plant secondary metabolites. Flavonoids were referred to as vitamin P probably because of the effect they had on the permeability of vascular capillaries. The flavonoids are a large group of naturally occurring phenylchromones found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. Up to several hundred milligrams are consumed daily in the average Western diet.

According to the IUPAC nomenclature, they can be classified into flavonoids or bioflavonoids, isoflavonoids, derived from 3-phenylchromen-4-one (3-phenyl-1, 4-benzopyrone) structure, neoflavonoids, derived from 4-phenylcoumarine (4-phenyl-1, 2-benzopyrone) structure. Three flavonoid classes above are all ketone-containing

compounds, and as such, are anthoxanthins (flavones and flavonols). This class was the first to be termed bioflavonoids. The terms flavonoid and bioflavonoid have also been more loosely used to describe non-ketone polyhydroxy polyphenol compounds which are more specifically termed flavonoids. The three cycle or heterocycles in the flavonoid backbone are generally called ring A, B, and C. Ring A usually shows a phloroglucinol substitution pattern [5]. Flavonoids are phenolic substances isolated from a wide range of vascular plants, with over 8000 individual compounds known. A variety of *in-vitro* and *in-vivo* experiments have shown that selected flavonoids possess antiallergic, anti-inflammatory, antiviral and antioxidant activities, significant anticancer activity including anti-carcinogenic properties and even a prodifferentiative activity, certain flavonoids possess potent inhibitory activity against a wide array of enzymes, but of particular note is their inhibitory effects on several enzyme systems intimately connected to cell activation processes such as protein kinase C, protein tyrosine kinases, phospholipase A2, and others [6, 7] potential antioxidants [8, 9] protective role against cardiovascular diseases [10, 11, 12, 13].

Recent analyses have focused on our understanding of the role of flavonoids in such well-established processes as plant-microbe interactions and protection against ultraviolet (UV) light, and have also uncovered a previously unsuspected role in male fertility [14]. They include pollinator attractants, oviposition stimulants, feeding attractants and deterrents, allelopathy and phytoalexins [15, 16]. Flavonoids protect plants against various biotic and abiotic stresses; flavonoid oxidation contributes to these chemical and biological properties and can lead to the formation of brown pigments in plant tissues as well as plant-derived foods and beverages [17, 18].

An Objective of Research: The objective of current research is to bring out the potential of flavonoids present in the plant which can be utilized as a commercial herbal drug to be used in therapies for various pathogenic microbes.

MATERIALS AND METHODS:

Plant Material: The *B. diffusa* was collected from the University of Rajasthan, Jaipur, Campus. A specimen was submitted to the Herbarium, Department of Botany, University of Rajasthan, Jaipur and the voucher specimen no. RUBL211299 was given. The plant material was shade dried, and different plant parts were collected separately, powdered and used as the experimental plant material for further experimentation.

Chemicals: All the chemicals used were of analytical grade and purchased from Hi-Media from Hi-media Laboratory Pvt. Ltd. Mumbai.

Tests for Flavonoids:

1) Shinoda's Test: To 2 ml of the test solution, a fragment of magnesium metal (mg^{++}) ribbon were added into the test tube, followed by the dropwise addition of concentrated conc. HCl. The resulting pink/scarlet/ crimson of occasionally green/blue colors indicated the presence of flavonoids [19].

2) NaOH Tests: To 2-3 ml of extract, few drops of sodium hydroxide solution were added into a test tube. Formation of intense yellow color that became colorless on the addition of a few drops of dilute HCl indicates the presence of flavonoids.

Flavonoids:

Extraction Procedure: Each of the dried and powdered test samples was Soxhlet for flavonoids using 80% methanol (100 ml/g dw; Subramanian and Nagarajan, 1969) for 24 h on a water bath. The methanolic extracts were filtered and concentrated *in vacuo* individually. Each of the residual syrup was fractionated by successive extraction (3 X) with petroleum ether (Fr. I), diethyl ether (Fr. II) and ethyl acetate (Fr. III). In each case, Fr. I was rejected due to its fatty components, whereas Fr. II and Fr. III were analyzed for free and bound-flavonoids respectively.

Fraction stored until analysis but, the Fr. III which contained flavonoids glycosides was acid-hydrolyzed (7% H_2SO_4; 10 ml/g) for 2 h. The hydrolysate was filtered, the filtrate was extracted with ethyl acetate (3X), which were later pooled, washed thoroughly with water till neutrality and concentrated under pressure. Its aqueous fraction was, however, not studied and discarded. Both the fraction II and III (the acid-hydrolyzed proteins) were constituted in ethanol, before chromato-graphic analysis and GC-MS analysis.

Chromatographic Analysis:

A) Thin Layer Chromatography (TLC) of Aglycones:

Qualitative: Thin glass plates coated with silica gel 'G' were air dried at room temperature. The dried plates were activated at 100 C for 15 min in an oven, cooled at room temperature and used to examine free and bound aglycones of each sample obtained. Each of the extracts was applied 1cm above the edge of the chromatographic plates along with the reference flavonoids used as a marker and developed in an airtight chromatographic chamber which has already been saturated with 200 ml of a solvent system of benzene- acetic acid - water (125: 72: 3) [20]. Several other solvent systems, such as *n*-butanol - acetic acid - water (4:1:5; upper phase), ethyl acetate saturated with water, acetic acid - water (6:4; 85:15), butanol - acetic acid water (TBA; 3:1:1) and Forestal system [acetic acid - conc. HCl - water, (10:3:30)] were also tried but benzene - acetic acid- water (125: 72: 3, solvent I) gave better separation.

Later, the developed chromatograms of each were exposed to I_2 vapors, UV light alone and in the presence of ammonia fumes (100 ml wide mouthed bottle containing conc. ammonia was held in close contact with each spot for 5-10 sec **Table 6**. The extracts resolved in to three spots (R_f 0.56, green yellow; 0.79, green yellow; 0.86, green yellow) were observed in daylight, and the spots coincided to the authentic markers, (1) Luteolin (R_f 0.56, green yellow) (2) Quercetin (R_f 0.79, green yellow) and (3) Kaempferol (R_f 0.86, green yellow).

Simultaneously, some of the developed chromatograms were also kept in I_2 chamber yel1owish-brown spots against a white background were observed. After evaporation of I_2 by continuous heating, few plates were sprayed with 5% alc. $FeCl_3$ as also with 1% alcoholic $AlCl_3$ separately and heated in an oven at 100°C for 5 min (Mabry *et al.*, 1970). Luteolin (R_f 0.56), quercetin (R_f 0.79) and kaempferol (R_f 0.86) and gave positive reactions to the spraying reagents [21, 22].

Preparative: Preparative TLC was performed on silica gel G coated plates, activated, cooled. The extract of *B. diffusa* and *T. undulata* along with authentic markers applied on the preparative TLC plates and developed in the solvent I. Such developed chromatograms coinciding to reference (visualized under UV light) were scrapped and eluted with ethyl acetate separately. Each of the eluates was dried over anhydrous Na_2SO_4, re-constituted in chloroform and crystallized using (methanol).

B) Identification: Each of the isolated compounds was subject its mp, UV, IR and NMR spectral studies with the authentic samples. Later, by the color reactions, TLC behavior and the spectrophotometric data, the isolated compounds were identified by comparing with that of the standards.

C) Quantification: The identified luteolin (L), quercetin (Q) and kaempferol (K) were quantitatively estimated by spectrophotometric methods [23, 24].

TABLE 1: CHROMATOGRAPHIC DATA AND COLOUR REACTION OF THE FLAVONOIDS ISOLATED FROM *BOERHAVIA DIFFUSA*

Flavonoids (aglycones)	R_f (×100) in						Colors by chromatogenic sprays color			
	BeAW$^+$	BAW*	TBA^{++}	Day- light	UV* ammonia	I_2vapours	$FeCl_3$		$AlCl_3$	
							Visible	UV	Visible	UV
Luteolin	56	84	77	GN-YW	YW	YW-BN	BN	BK	DL-YW	YW-GN
Quercetin	79	64	41	GN-YW	YW	YW-BN	BT-GY	BK	DL-YW	YW-GN
Kaempferol	86	83	55	GN-YW	YW-BN	YW-BN	BN	BK	YW	YW-GN

TABLE 2: ISOLATED FLAVONOID CONTENT (mg/g dw*) IN *BOERHAVIA DIFFUSA*

Plant species	Free (F)				Bound (B)				F+B			
	Quercetin	Kaempferol	Luteolin	Total	Quercetin	Kaempferol	Luteolin	Total	Quercetin	Kaempferol	Luteolin	Total
B. diffusa												
Root	0.07	0.10	0.08	0.25	0.04	0.08	0.05	0.17	0.11	0.18	0.13	0.42
Stem	0.08	0.09	0.06	0.23	0.05	0.07	0.04	0.16	0.13	0.16	0.10	0.39
Leaves	0.15	0.20	0.08	0.43	0.10	0.12	0.03	0.25	0.25	0.32	0.11	0.68

Stock solutions of L, Q and K were prepared in methanol (1 mg/ml), out of which varied concentrations (20 ug to 160 ug) were separately spotted on TLC plates, developed above, air-dried and visualized under UV light as also I_2 vapors. The spots marked on the basis or fluorescence was

collected along the absorbent in separate test tubes. Later, to every 5 ml of spectroscopic methanol was added shaken vigorously, centrifuged and the supernatants were collected separately. The volume of each was raised to 10 ml by methanol, to which 3 ml of 0.1 M $AlCl_3$ solution was added by vigorous shaking and kept at room temperature for 20 min. The OD of each of the sample was taken on a spectrophotometer set at 426 for L, 424 nm for K and 440 nm for Q and the average of five replicates of each was calculated. A regression curve for each of that authentic compound (L, Q, K) was plotted in between the various concentrations and their respective ODs, which followed the Beer is Law.

Likewise, each of the free (F) and bound (B) fractions of were dissolved in 1 ml of methanol, spotted (0.1 ml) on TLC along with the authentic samples and the fluorescent spots coinciding with those of the authentic markers were marked, scrapped, eluted and processed as mentioned above. The ODs were recorded and the level of flavonoids in each was computed (mg/g dw) from the standard calibration curves. Three such replicates were run and their average value was recorded.

GC-MS Analysis:

GC-MS Conditions: GCMS-QP 2010 Plus was used for identification and quantification of phytoconstituents, using MS libraries previously compiled from purchased standards. For the acquisition of an electron ionization mass spectrum, an ion source temperature of 250 °C was used. The GC was equipped with an SE-30 capillary column a split injection piece (270 °C) and direct GC-MS coupling (280 °C). Helium (1.2 mL/min) was used as the carrier gas with a split ratio of 1:10. The oven temperature program for analyzing the extracts utilized an initial oven temperature of 100°C, maintained for 2 min, followed by a steady climb to 200 °C at a rate of 7 °C/min allowed to increase to 190 °C at a rate of 30 °C/min. This oven temperature was again maintained at 190 °C for 5 min and then allowed to increase to 300 °C at a rate of 7 °C/min. This oven temperature was maintained for 2 min and finally ramped to 300 °C at a rate of 10 °C/min and maintained for a further 22 min. Injection temperature was 270° C and volume 250 °C and 1 µl, respectively. The total GC running time was about 43.28 min. The MS operating conditions were as follows, interference temperature of 260 °C, ion source temperature of 250 °C, mass scan (m/z)-40-450, solvent cut time 7 min, scan speed 2000 amu/s total MS running time-50.28 min and Threshold -1000.

Identification: GC-MS is a valuable aid for identifying unknown peak as well as for confirming the identification of identified phytoconstituents. In some cases when no identical spectra were found, the structural type of the corresponding component was suggested only by its mass spectral fragmentation and retention data. Identification of components was based on directs comparison of the retention times and mass spectral data with those for standard compounds and computer matching with the library (Wiley library, NIST data bank, database NIST 98) as well as by comparison of the retention time.

Sources of Test Organisms:

Fungi: The fungal strains *Aspergillus niger* (NCIM 0616), *Fusarium oxysporium* (NCIM 1228), *Trichoderma reesei* (NCIM 0992), *Penicillium funiculosum* (NCIM 1075), *Candida albicans* (NCIM- 3501), *Trichoderma viride* are procured from the National Institute for Complementary Medicine.

Bacteria: The bacterial strains *Escherichia coli* (MTCC 1652), *Staphylococcus aureus* (MTCC 0087) (Gram +ve), *Pseudomonas aeruginosa* (MTCC 4646) (Gram +ve), *Bacillus subtilis* (MTCC 0121), *Klebsiella pneumoniae* (MTCC-0109) (Gram –ve) and *Streptomyces albudencus* (MTCC 1764), *Enterococcus faecalis* (ATCC-29212) (Gram +ve) were procured from the microbial type culture collection (Institute of Microbial Technology, Chandigarh, India).

Culture of Test Microbes: For the cultivation of bacteria, nutrient broth medium (NB) was prepared using 8% nutrient broth (Difco) in distilled water and agar-agar and sterilized at 15 lbs psi for 25-30 min. Agar test plates were prepared by pouring ~15 ml of NBM into the petri dishes (10 mm) under aseptic conditions. A peptone saline solution was prepared (by mixing 3.56 g KH_2PO_4 + 7.23 g NaH_2PO_4 + 4.30 g, NaCl + 1 g peptone in 1000 ml

of distilled water, followed by autoclaving) and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37 °C for 24 h. However, for the cultivation of fungi, potato dextrose agar (PDA) medium was prepared by mixing 100 ml potato infusion + 20 g agar + 20 g glucose, followed by autoclaving) and the test fungi were incubated at 27 °C for 48 h and the cultures were maintained on same medium by regular subculturings.

Fungicidal and Bactericidal Assay: For both, fungicidal and bactericidal assays agar well diffusion method was adopted [25], because of reproducibility and precision. The different test organism were proceeded separately using a sterile swab over previously sterilized culture medium plates, and the zone of inhibition were measured around wells in solidified medium (5 mm in diameter), which were containing 2 mg/ml and 4 mg/ml of the test extracts, control solvent or streptomycin (1 mg/ml) or ketoconazole (1 mg/ml) as reference separately.

These plates were initially placed at low temperature for 1 h, so as to allow the maximum diffusion of the compounds from the wells into the plate and later, incubated at 37 °C for 24 h in case of bacteria and 48 h at 27 °C for fungi, after which the zones of inhibition could be easily observed. Three replicates of each test extract were examined, and the mean values were then referred.

RESULTS AND DISCUSSION: Amongst the free form of flavonoids extracted from *B. diffusa*, in roots kaempferol was obtained in maximum mount while quercetin is observed in minimum amount. (in roots; kaempferol; 0.10 mg/g dw>luteolin; 0.08 mg/g dw > quercetin; 0.07 mg/g dw), in stems kaempferol is maximum while luteolin is minimum (kaempferol; 0.09 mg/g dw > quercetin; 0.08 mg/g dw > luteolin; 0.06 mg/gdw), in leaves kaempferol is observed in highest amount and luteolin in lowest amount (kaempferol; 0.20 mg/g dw > quercetin; 0.15 mg/g dw > luteolin; 0.08 mg/g dw) maximum amount of total free flavonoids was observed in leaves (leaves; 0.43 mg/g dw > roots; 0.25 mg/g dw > stems; 0.23 mg/g dw), amongst the bound form of flavonoids in roots kaempferol was reported in maximum amount while quercetin was observed in minimum amount (kaempferol; 0.08 mg/g dw > luteolin; 0.05 mg/g dw > quercetin; 0.04 mg/g dw) while in stems and leaves kaempferol was observed in higher amount while luteolin was observed in lower amount (kaempferol; 0.07 mg/g dw > quercetin; 0.05 mg/g dw > luteolin; 0.04 mg/g dw) (in leaves; kaempferol; 0.12 mg/g dw> quercetin; 0.10 mg/g dw > luteolin; 0.03 mg/gdw).

Maximum amount of total bound form of flavonoids was observed in leaves (leaves; 0.25 mg/g dw > root; 0.17 mg/g dw > stems; 0.16 mg/g dw). The total flavonoid content (F+B) was observed maximum in leaves and minimum in stems (leaves; 0.68 mg/g dw > root; 0.42 mg/g dw > stems; 0.39 mg/g dw). Other various kinds of flavonoids have also been isolated in significant quantities from *Boerhavia erecta* which have also been screened for their pharmacological activities. A higher content of flavonoids and their antioxidant activities have been reported in *Boerhavia* [26, 27].

Root extracts of *Boerhavia species* have been reported to have a higher amount of flavonoid content, which also supports its significant antioxidant activity. Higher quercetin levels in aerial parts in *Boerhavia diffusa* are responsible for various pharmacological activities and are used in various formulations [28]. The eluted compounds from TLC were pooled together according to their TLC behavior and isolate them with the solvents and evaporated yielding three flavonoids kaempferol, quercetin, and luteolin. The spectral analyses **Table 3** of the active constituent, (a) Luteolin (b) Quercetin and (c) Kaempferol from the different plant parts of *Boerhavia diffusa* and *Tecomella undulata* are shown below: -

Luteolin: Yellow needles on crystallization (mp 280°-320°C). UV light absorption MeOH: 242 sh, 253 sh, 267 sh, 291 sh, 349 sh. IR: νcm^{-1}/ max KBr: 3400, 3423, 3100 (O–H), 1070, 1150, 1010 (C=O), 1656, 1620, 1612 (C=C), 1514 (aromatic), 1103, 1862, 1839, 1562. ^{1}HNMR (300MHz, $CDCl_3$): 3.42, (H_1), 3.49 (H_2), 3.56 (H_3), 6.30 (H_4), 3.68 (H_5), 3.85 (H_6), 5.10 (H_7), 6.63 (H_8), 6.83 (H_9), 6.95(H_{10}), 7.41(H_{11}), 7.43(H_{12}). ^{13}C NMR (300MHz, $CDCl_3$): 122.6 (C_1), 113.8 (C_2), 76.8 (C_3), 70.3 (C_4), 77.4 (C_5), 100.5 (C_6), 163.9 (C_7), 95.8 (C_8), 158.0(C_9), 106.3 (C_{10}), 165.8 (C_{11}), 146.3(C_{12}), 150.4 (C_{13}), 121.1 (C_{14}), 119.0 (C_{15}).

Quercetin: Yellowish needles on crystallization (mp 312°-313°C). UV light absorption MeOH: 255 sh, 301 sh, 374 sh, 440 sh. IR: νcm^{-1}/ max KBr: 3420, 3380(O–H), 2800 (C-H), 2100 (C=C), 1680 (C=O), 1610 (C≡C), 1560, 1510, 1450, 1400 (aromatic), 1385, 1310, 1270, 1180, 1010. ^{1}HNMR (300MHz, $CDCl_3$): 2.45, (H_1), 2.55 (H_2), 6.79 (H_3), 6.98 (H_4), 6.49 (H_5), 2.33 (H_6), 6.38 (H_7), 2.36 (H_8), 5.37 (H_9), 1.4 (H_{10}). ^{13}C NMR (300MHz, $CDCl_3$): 137.3 (C_1), 137.9 (C_2), 14.2 (C_3), 127.0 (C_4), 126.1 (C_5), 133.8 (C_6), 142.4 (C_7), 158.2 (C_8), 114.6(C_9), 134.5 (C_{10}), 123.0 (C_{11}), 138.0 (C_{12}), 121.1 (C_{13}), 149.4 (C_{14}), 108.9 (C_{15}), 127.8.

Kaempferol: Brownish needles on crystallization (mp 312°-313°C). UV light absorption MeOH: 253 sh, 269 sh, 305 sh, 374 sh, 424 sh. IR: νcm^{-1}/ max KBr: 3420 (O–H), 2830 (C-H), 2240 (C=C), 1700 (C=O), 1600, 1610 (C≡C), 1560, 1510, 1450, 1400 (aromatic), 1385, 1310, 1270, 1180, 1010, 815. ^{1}HNMR (300MHz, $CDCl_3$): 2.35(H_1), 7.01(H_2), 7.18 (H_3), 6.29 (H_4), 6.37 (H_5), 2.35 (H_6), 5.39 (H_7), 5.36 (H_8), 7.18 (H_9), 7.01 (H_{10}). ^{13}C NMR (300MHz, $CDCl_3$): 1.36 (C_1), 129.8 (C_2), 126.8 (C_3), 131.9 (C_4), 147.4 (C_5), 154.2 (C_6), 114.6 (C_7), 137.5 (C_8), 124.0 (C_9), 136.0 (C_{10}), 121.1 (C_{11}), 149.4 (C_{12}), 106.9 (C_{13}), 131.9 (C_{14}), 126.1 (C_{15}).

TABLE 3: SPECTRAL STUDIES OF ISOLATED FLAVONOIDS FROM *BOERHAVIA DIFFUSA*

Name of Compound	UV light absorption MeOH	IR: νcm^{-1}/ max KBr	^{1}H NMR	^{13}C NMR
Luteolin	242 sh, 253 sh, 267 sh, 291 sh, 349 sh	3400, 3423, 3100 (O–H), 1070, 1150, 1010(C=O), 1656, 1620, 1612 (C=C), 1514 (aromatic), 1103, 1862, 1839, 1562	3.42, (H_1), 3.49 (H_2), 3.56 (H_3), 6.30 (H_4), 3.68 (H_5), 3.85 (H_6), 5.10 (H_7), 6.63 (H_8), 6.83 (H_9), 6.95(H_{10}), 7.41(H_{11}), 7.43(H_{12})	13C-NMR (100 MHz, Acetone-*d6*): d 182.4 (C4), 164.5 (C7), 164.2 (C2), 162.7 (C5), 158.1 (C9), 149.4 (C4'), 145.8 (C3'), 123.1 (C1), 119.5 (C6), 116.0 (C5), 113.5 (C2), 104.7 (C10), 103.6 (C3), 99.0 (C6), 94.0 (C8).
Quercetin	255 sh, 301 sh, 374 sh, 440 sh	3420, 3380(O–H), 2800 (C-H), 1680 (C=O), 1610, 1610, 1560, 1510, 1450, 1400 (aromatic), 1385, 1310, 1270, 1180, 1010	2.35, (H_1), 2.35 (H_2), 6.89 (H_3), 6.99 (H_4), 6.29 (H_5), 2.35 (H_6), 6.37 (H_7), 2.35 (H_8), 5.39 (H_9), 5.36 (H_{10})	138.3 (C_1), 137.6 (C_2), 14.4 (C_3), 129.0 (C_4), 123.1 (C_5), 131.8 (C_6), 147.4 (C_7), 154.2 (C_8), 114.6(C_9),137.5 (C_{10}), 124.0 (C_{11}), 136.0 (C_{12}), 121.1 (C_{13}), 149.4 (C_{14}), 106.9 (C_{15}), 126.8
Kaempferol	253 sh, 269 sh, 305 sh, 374 sh, 424 sh	3420 (O–H), 2830 (C-H), 1700 (C=O), 1600, 1610, 1560, 1510, 1450, 1400 (aromatic), 1385, 1310, 1270, 1180, 1010, 815	2.35(H_1), 7.01(H_2), 7.18 (H_3), 6.29 (H_4), 6.37 (H_5), 2.35 (H_6), 5.39 (H_7), 5.36 (H_8), 7.18 (H_9), 7.01 (H_{10})	1.36 (C_1), 129.8 (C_2), 126.8 (C_3), 131.9 (C_4), 147.4 (C_5), 154.2 (C_6), 114.6 (C_7), 137.5 (C_8), 124.0 (C_9), 136.0 (C_{10}), 121.1 (C_{11}), 149.4 (C_{12}), 106.9 (C_{13}), 131.9 (C_{14}), 126.1 (C_{15})

A GC-MS analysis of the extracted flavonoids from various plant parts of *Boerhavia diffusa* namely root, stem and leaf was carried out. Various constituents obtained are reported.

Antifungal activity of the flavonoids extracted from different plant parts when tested against *Fusarium* showed that root showed maximum inhibition while stem showed minimum inhibition (Root; IZ=29 ± 1 mm > leaf; IZ= 18.33 ± 1.52 mm > Stem; IZ=12.33 ± 1.15 mm). When tested against *Penicillium funiculosum*, leaf showed maximum inhibition while stem showed mini (Leaf; IZ=16.33 ± 1.53 mm > root; IZ= 15.5 ± 1.32 mm > Stem; IZ=10.33 ± 1.15 mm). Against *Candida albicans* root extract showed maximum inhibition while stem showed minimum (Root; IZ=18.16 ± 1.04 mm > leaf; IZ= 12.67 ± 1.52 mm > Stem; IZ=12.00 ± 2.00 mm). Against *T. virdae*, leaf showed maximum inhibition while root showed minimum inhibition. (Leaf; IZ=26.00 ± 2.00 mm > stem; IZ= 15.66 ± 0.57 mm > root; IZ=14.00 ± 0.5 mm).

Antibacterial activity of flavonoids against S. *aureus* was shown maximum by root and stem whereas minimum by leaf (root, stem; IZ=25.66 ± 0.57 mm > leaf; IZ=24.34 ± 2.08 mm). While against *E. coli* leaf showed maximum activity and root showed minimum inhibition (Leaf; IZ=27.00 ± 1.00 mm > stem; IZ= 18.00 ± 1.00 mm > root; IZ=14.33 ± 1.15 mm). Against *Enterococcus*, leaf showed maximum activity and stem showed minimum (Leaf; IZ=9.67 ± 0.57 mm > root; IZ= 8.66 ± 1.15 mm > Stem; IZ= 0.00 mm). Against *Bacillus subtilis*, leaf showed maximum activity and root showed minimum activity (Leaf; IZ=16.33 ± 1.52 mm > stem; IZ= 14.66 ± 0.57 mm > root; IZ=14.33 ± 1.54 mm).

Against *Klebsiella pneumonia*, leaf and stem showed maximum whereas root showed minimum inhibitory activity (Leaf, stem; IZ=13.33 ± 2.08 mm > root; IZ= 12.33 ± 0.57 mm). Flavonoids, phytosterols and alkaloids crude extracts of root stem and leaves of *Boerhavia diffusa* in the present

study were evaluated for their antibacterial and antifungal efficacy. In this study, flavonoids from leaf extract showed highest inhibition zone against both test fungi and bacteria.

TABLE 4: BACTERICIDAL AND FUNGICIDAL EFFICACY OF FLAVANOIDS OF *BOERHAVIA DIFFUSA*

Microorganisms				
Fungi		**Root**	**Stem**	**Leaf**
F. oxysporium	IZ	20.00±1	12.33±1.15	29.33±1.52
NCIM 1228	AI	1.18	0.51	0.67
P. fumiculosum	IZ	15.5±1.32	10.33±1.15	16.33±1.53
NCIM 1075	AI	0.71	0.50	0.78
C. albicans	IZ	18.16±1.04	12.00±2	12.67±1.52
NCIM 3501	AI	0.98	0.71	0.75
T. viridie	IZ	14±0.5	15.66±0.57	26.00±2
NCIM	AI	0.43	0.53	0.96
Bacteria				
S. aureus	IZ	25.66±0.57	24.66±0.57	25.66±2.08
MTCC 0087	AI	0.86	0.86	0.81
E. coli	IZ	21.00±1.15	18.00±1	30.00±1
MTCC 1652	AI	0.8	1.00	1.50
E. faecalis	IZ	8.66±1.15	0.00	9.67±0.57
ATCC 29212	AI	0.43	0.00	0.49
B. subtilis	IZ	14.33±1.54	14.66±0.57	16.33±1.52
MTCC 0121	AI	0.72	0.73	0.82
K. pneumonia	IZ	12.33±0.57	13.33±1.52	13.33±2.08
MTCC 0109	AI	0.77	0.83	0.83

IZ = Inhibition zone (in mm) including the diameter of well (5 mm), Activity index = Inhibition area of the test sample/ Inhibition area of the test Standards. Standards: Zentamycin = 1.0 mg/ml; Gentamycin = 1.0 mg/ml. Results are mean value SD from atleast three experiment, S.E. ($\sigma\text{-}_x = \sigma / n^{1/2}$), σ = Standard deviation, n = no. of set.

TABLE 5: RETENTION TIME, MOLECULAR WEIGHT AND % AREA BY SETTING THE TOTAL PEAK AREA TO 100% OF FLAVONOIDS IDENTIFIED BY GC-MS IN LEAVES OF *BOERHAVIA DIFFUSA*

Peak #	**R. Time**	**Area%**	**Name**	**Mol. Formula**	**Mol. Wt**
1	9.318	4.57	Ethanone, 1-(4-chlorophenyl)-	C_8H_7ClO	154
2	18.355	5.39	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6-hy	$C_{11}H_{16}O_3$	196
3	19.008	0.75	Butyric acid, m-methoxyphenyl ester	$C_{11}H_{14}O_8$	194
4	19.201	0.26	5-Undecene, 3-methyl-, (Z)-	$C_{12}H_{24}$	168
5	19.291	1.79	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
6	19.381	0.58	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280
7	19.65	0.37	Butanoic acid, 3,7-dimethyl-6-octenyl ester	$C_{14}H_{26}O_2$	226
8	19.92	0.86	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
9	20.571	3.09	Methyl 17,18-dideuteriooctadecanoate	$C_{19}H_{36}D_2O_2$	300
10	21.522	2.11	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
11	22.905	0.57	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
12	22.977	2.1	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296
13	23.174	0.66	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*	$C_{20}H_{40}O$	296
14	23.293	0.6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	
15	23.77	0.68	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280
16	23.832	2.86	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310
17	24.145	1.03	Octadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	312
18	24.629	0.26	Piperidine-1-dithiocarboxylic acid, 2-oxocyclopentyl ester	$C_{11}H_{17}NOS_2$	243
19	25.786	0.17	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298
20	26.657	1.55	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370
21	26.794	1.56	5-(4-Chlorophenyl)-2-(1-piperidinyl)-6h-1,3,4-th	$C_{14}H_{16}ClN_3S$	293
22	28.398	0.55	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340
23	28.822	8.02	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390
24	29.463	2.72	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368
25	32.211	0.36	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284
26	35.809	0.32	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312
27	36.675	0.79	Cholesta-4,6-dien-3-ol, (3.beta.)-	$C_{27}H_{44}O$	384
28	37.032	0.17	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430
29	38.075	0.81	(-)-5-Oxatricyclo[8.2.0.0(4,6)]dodecane,,12-trime	$C_{15}H_{24}O$	220
30	38.306	0.19	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	466
31	38.592	7.72	.beta.-Sitosterol	$C_{29}H_{50}O$	414

TABLE 6: RETENTION TIME, MOLECULAR WEIGHT AND % AREA BY SETTING THE TOTAL PEAK AREA TO 100% OF FLAVONOIDS IDENTIFIED BY GC-MS IN STEMS OF *BOERHAVIA DIFFUSA*

Peak#	R. Time	Area%	Name	Mol. Formula	Mol. Wt
1	17.211	6.39	beta.-d-Mannofuranoside, methyl	$C_7H_{14}O_6$	194
2	17.673	0.65	Dihexylsulfide	$C_{12}H_{26}S$	202
3	19.234	2.75	1-Decene, 8-methyl-	$C_{11}H_{22}$	154
4	19.323	4.46	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
5	19.688	0.57	2-Decen-1-ol	$C_{10}H_{20}O$	156
6	19.956	1.76	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
7	20.605	8.20	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
8	21.278	0.72	Phthalic acid, 4-bromophenyl heptyl ester	$C_{21}H_{23}BrO_4$	418
9	21.534	11.3	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
10	22.922	1.7	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322
11	22.987	6.69	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268
12	23.774	1.9	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280
13	23.835	6.3	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310
14	26.575	0.79	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312
15	27.718	0.4	Cyclohexanecarboxylic acid, heptadecyl ester	$C_{24}H_{46}O_2$	366
16	27.898	0.74	4-Undecene, 9-methyl-, (Z)-	$C_{12}H_{24}$	168
17	28.407	1.96	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340
18	28.845	2.17	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390
19	29.468	2.11	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312
20	32.227	1.26	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284
21	36.325	0.28	trans-Z-.alpha.-Bisabolene epoxide	$C_{15}H_{42}O$	220
22	36.377	0.6	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436
23	36.557	0.8	Pseduosarsasapogenin-5,20-dien	$C_{27}H_{42}O_3$	414
24	36.677	1.99	Cholesta-4,6-dien-3-ol, benzoate, (3.beta.)-	$C_{34}H_{48}O_2$	488
25	36.833	20.66	.beta.-Sitosterol	$C_{29}H_{50}O$	414
26	37.315	1.33	Ethyl nonadecanoate	$C_{21}H_{42}O_2$	326
27	38.304	0.75	Triacontanoic acid, methyl ester	$C_{31}H_{26}O_2$	466
28	38.773	1.45	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368
29	39.85	0.85	Pregn-4-ene-3,20-dione	$C_{21}H_{30}O_2$	314

TABLE 7: RETENTION TIME, MOLECULAR WEIGHT AND % AREA BY SETTING THE TOTAL PEAK AREA TO 100% OF FLAVONOIDS IDENTIFIED BY GC-MS IN ROOTS OF *BOERHAVIA DIFFUSA*

Peak#	R. Time	Area%	Name	Mol. Formula	Mol. Wt
1	17.685	0.41	1-Undecanol	$C_{11}H_{24}O$	172
2	19.414	1.01	1-Undecene, 9-methyl-	$C_{12}H_{24}$	168
3	19.958	1.01	Butanoic acid, 3,7-dimethyl-6-octenyl ester	$C_{14}H_{26}O_2$	226
4	20.611	5.04	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
5	21.288	0.63	1,2-Benzenedicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$	278
6	21.538	6.51	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
7	22.924	0.8	7-Tetradecyne	$C_{14}H_{26}$	194
8	22.99	3.49	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296
9	23.776	1.11	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280
10	23.835	5.47	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310
11	24.955	0.31	Bicyclo[2.2.1]heptan-2-ol, 6-methyl-6-nitro	$C_{18}H_{13}NO_3$	171
12	25.799	0.42	Nonanoic acid, 7-methyl-, methyl ester	$C_{11}H_{22}O_2$	186
13	27.548	17.77	1,2-Propanediol, 3-benzyloxy-1,2-diacetyl-	$C_{14}H_{18}O_5$	226
14	28.829	1.56	1,2-Benzenedicarboxylic acid, diisooctylest	$C_{24}H_{38}O_4$	390
15	29.242	13.27	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	$C_{22}H_{20}O_5$	332
16	31.416	0.22	Nonanoic acid, 2,4,6-trimethyl-, methyl ester, (2S,4S,6R)-(+)-	$C_{13}H_{26}O_2$	214
17	32.216	1.12	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382
18	33.491	1.71	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312
19	35.145	0.38	Hexatriacontane	$C_{36}H_{74}$	506
20	35.808	2.14	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368
21	36.06	0.61	2-Bromotetradecane	$C_{14}H_{29}Br$	276
22	36.2	0.06	1,E-11,Z-13-Octadecatriene	$C_{18}H_{32}$	248
23	36.375	0.27	Stigmasterol	$C_{29}H_{48}$	248
24	36.551	0.96	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{14}H_{22}O$	206

25	36.603	0.21	1-Octanol, 2-butyl-	$C_{12}H_{26}O$	186
26	36.671	1.15	Cholesta-4,6-dien-3-ol, benzoate, (3.beta.)-	$C_{34}H_{48}O_2$	488
27	36.829	13.39	.beta.-Sitosterol	$C_{29}H_{50}O$	414
28	37.173	1.12	Benzene, 1,1'-[1-(2,2-dimethyl-3-butenyl)-1,3-propanediyl]bis-	$C_{21}H_{26}$	278
29	37.902	2.60	Tetratriacontane	$C_{34}H_{70}$	478
30	38.075	0.98	2,4a,8,8-Tetramethyldecahydrocyclopropa[d]naphthalene	$C_{15}H_{26}$	206
31	38.773	0.64	Heptadecanoic acid, ethyl ester	$C_{19}H_{38}O_2$	298
32	40.808	0.28	Hexanoic acid, 2-tetradecyl ester	$C_{20}H_{40}O_2$	312

CONCLUSION: *Boerhavia diffusa* is a perennial herb, belongs to family Nyctaginaceae. Grows as a common weed, commonly known as punarnava, it is distributed in Tropical parts of the world. It is rich in phytochemicals lignins, carbohydrates, lipids, proteins, ascorbic acid, glycoproteins, phenolic compounds, flavonoids, sterols, and alkaloid. It shows a good inhibitory activity against pathogenic fungi and gram-negative and gram-positive bacteria [29, 30, 31]. Their mode of antibacterial activity may be due to cell lysis and disruption of the cytoplasmic membrane upon membrane permeability [32]. It also has potent pharmacological activities [34].

With all the experiments and investigations performed in the present study, we conclude that the plant studied in the present study *Boerhavia diffusa* have good amount of phytochemicals and these phytochemicals are responsible for imparting properties like antimicrobial and antioxidant to these plants. It also show good inhibitory activity against pathogenic bacteria and fungi tested, this result thus forms a platform for further study of the phytochemicals, bioassays to identify single molecules from plants that have interesting bioactivities in isolation and might be useful lead compounds for the development of pharmaceutical drugs as antibiotics against certain infections caused by these bacteria and fungi with enhanced activity and reduced toxicity.

The plant also shows good antioxidant activity due to which these plants can also be studied for their potential against the diseases caused by free radicals.

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REFERENCES:

1. Koh YC and Pan MH. Review on discovery and development of novel phytochemicals which can be used in functional foods. Current Research in Nutrition and Food Science 2018; 6(2): 241-62.
2. Grierson DS and Afolayan AJ: Antibacterial activity of some indigenous plants used for the treatment of a wound in the Eastern Cape; South Africa. J of Ethno-pharmacology 1999; 66: 103-06.
3. Douglas NA and Manos PS: Molecular phylogeny of Nyctaginaceae: taxonomy, biogeography, and characters associated with radiation of xerophytic genera in North America. Am J Bot 2007; 94(5): 856-72
4. Kumar R, Gautam S, Singh KD, Kumar P, Haque N, Yadav V, Kumar R, Diwakar RP, Rishikant and Kumar SB: Pharmacological properties of *Boerhavia diffusa*: A review. International Journal of Chemical Studies 2018; 4: 72-80.
5. Wang TY, Li Q and Bi KS: Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. Asian Journal of Pharmaceutical Sciences 2018; 13: 12-23.
6. Tungmunnithum D, Thongboonyou A, Pholboon A and Yangsabai A: Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines 2018; 5(93): 1-16.
7. Umesh CV, Jamsheer AM, Prasad M A: The role of flavonoids in drug discovery- review on potential applications. Research Journal of Life Sciences, Bio-informatics, Pharmaceutical and Chemical Sciences 2018; 4(1): 70-77.
8. Bhardwaj R, Yadav A and Sharma RA: *Tecomella undulata*-phenolic compounds and antioxidant activities. Research Journal of Medicinal Plants 2014; 8(5): 223-30.
9. Bhardwaj R, Yadav A, Sharma RA and Sharma P: Anti-oxidant properties of methanolic extracts of *Boerhavia diffu*sa. Research J of Phytochemistry 2014; 8(3): 119-26.
10. Zhao L, Wang JL, Liu R, Li XX, Li JF andZhang L: Neuroprotective, anti-amyloidogenic and neurotrophic effects of apigenin in an Alzheimer's disease mouse model. Molecules 2013; 18: 9949-65.
11. Castell M, Perez-Cano FJ, Abril-Gil M and Franch A: Flavonoids on allergy. Curr Pharm Des 2014; 20: 973-87.
12. González-Gallego J, García-Mediavilla MV, Sánchez-Campos S and Tuñón MJ: Fruit polyphenols, immunity and inflammation. Br J Nutr 2010; 104(3): 15-27.

13. Kozłowska A and Szostak-Wegierek D: Flavonoids--food sources and health benefits. Rocz Panstw Zakl Hig 2014; 65(2): 79-85.
14. Shirley BW: Flavonoid biosynthesis: 'new' functions for an 'old' pathway. Trends in Plant Science 1996; 1(11): 377-82.
15. Iwashina T: Flavonoid function and activity to plants and other organisms, BiolSci Space 2003; 17(1): 24-44.
16. Du Y, Chu H, Wang M, Chu IK and Lo C: Identification of flavone phytoalexins and a pathogen-inducible flavone synthase II gene (SbFNSII) in sorghum. J Exp Bot 2010; 61(4): 983-94.
17. Agati G, Azzarello E, Pollastri S and Tattini M: Flavonoids as antioxidants in plants: Location and functional significance. Plant Science 2012; 196: 67-76.
18. Hoensch HP and Oertel R: The value of flavonoids for the human nutrition: Short review and perspectives. Clinical Nutrition Experimental 2015; 3: 8-14.
19. Maríaa R, Shirleya M, Xavierc C, Jaimea S, Davida V, Rosad S and Jodie D: Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. Jour of King Saud University-Science 2018; 30: 500-05.
20. Wong E and Francis CM: Flavonoids in genotypes of *Trifolium subterraneum* I. The normal flavonoid pattern of the gerald ton variety. Phytochemistry 1968; 7: 2123-2129.
21. Gwatidzo L, Dzomba P and Mangena M: TLC separation and antioxidant activity of flavonoids from *Carissa bispinosa*, *Ficus sycomorus*, and *Grewia bicolar* fruits. Nutrire 2018; 43: 3.
22. Dhaniya S and Parihar SK: Isolation and identification of flavonoids from *Alhagi maurorum*. J of Pharmacognosy and Phytochemistry 2018; 7(3): 1321-25.
23. Struchkov P, Beloborodov V, Kolkhir V, Voskoboynikova I and Savvateev A: Comparison of spectrophotometric methods of total flavonoid assay based on complex formation with aluminum chloride as applied to multicomponent herbal drug angionorm. Journal of Pharmaceutical Negative Results 2018; 9(1): 1-7.
24. Ramos RTM, Bezerra ICF, Ferreira MRA and Soares LAL: Spectrophotometric quantification of flavonoids in herbal material, crude extract, and fractions from leaves of *Eugenia uniflora* Linn. Pharmacognosy Research 2017; 9(3): 253-60.
25. Bereksi MS, Hassaïne H, Bekhechi C and Abdelouahid DE: Evaluation of antibacterial activity of some medicinal plants extracts commonly used in Algerian traditional medicine against some pathogenic bacteria. Pharmacognosy Journal 2018; 10(3): 507-12.
26. Khalid M, Siddiqui HH and Fareed S: *In-vitro* estimation of the antioxidant activity and phytochemical screening of *Boerhavia diffusa* root extract. Asian Journal of Traditional Medicines 2011; 6(6): 259-66.
27. Ammar AF, Zhang H and Siddeeg A: *In-vitro* antioxidant activity and total phenolic and flavonoid contents of alhydwan (*Boerhavia elegana* Choisy) Seeds. Journal of Food and Nutrition Research 2014; 2(5): 215-20.
28. Mishra S, Aeri V, Gaur K, Sanjay M and Jachak: Phytochemical, therapeutic, and ethnopharmacological overview for a traditionally important herb: *Boerhavia diffusa* Linn. BioMed Research International 2014; 1-19.
29. Bhardwaj R: GC-MS analysis and antimicrobial activity of alkaloids of *Tecomella undulata*. Journal of Medicinal Plant Studies 2018; 6(6): 68-72.
30. Nobakht M, Trueman SJ, Wallace HM, Brooks PR, Streeter KJ and Katouli M: Antibacterial properties of flavonoids from kino of the eucalypt tree, *Corymbia torelliana*. Plants 2017; 6: 39.
31. Moloney MG: Natural products as a source for novel antibiotics. Trends Pharmacol Sci 2016; 37: 689-01.
32. Tagousop CN, Ekom JDTSE, Ngnokam D and Voutquenne-Nazabadioko L: Antimicrobial activities of flavonoid glycosides from *Graptophyllum grandulosum* and their mechanism of antibacterial action. BMC Complementary and Alternative Medicine 2018; 18: 252.
33. González-Gallego J, García-Mediavilla MV, Sánchez-Campos S and Tuñón MJ: Anti-inflammatory and immunomodulatory properties of dietary flavonoids. Polyphenols in Human Health and Disease. Polyphenols in Human Health and Disease 2014; 1: 435-52.

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