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Original Article

Determination of Polyphenolic content and Antioxidant Activity from Various Extracts of *Boerhaavia diffusa* Linn Root: An *In Vitro* Approach for Selection of Appropriate Extracting Solvent

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ABSTRACT

Objective: This study is aimed to evaluate extraction capacity of different solvents (toluene, dichloromethane, chloroform, ethyl acetate, ethanol, methanol, water, 60% aqueous methanol, 60% aqueous ethanol and 60% aqueous acetone) and its effect on total phenolic content, total flavonoid content, and antioxidant assay. **Methods**: Extraction was performed from dried root powder of *Boerhavia diffusa* using various solvents at 25˚C on magnetic sirr (300 rpm). Extraction yield, total phenolic content, total flavonoid content and total tannin content of the extracts were determined spectrophotometrically using gallic acid, quercetin as standards. Antioxidant potential determines by using various *in vitro* methodologies such as DPPH, FRAP, and ABTS assay. **Result**: The 60% aqueous methanol showed the highest extracting yield, in contrast, toluene and hexane showed the lowest yield. Highest total phenolic content (239.8±0.25 mg GAE/g) and total flavonoid content (131.1±4.20 mg QCE/g), were found from methanolic extract. While, acetone extract showed highest tannin content. The Methanolic extract of *Boerhaavia diffusa* exhibited the highest antioxidant activity. **Conclusions:** The highest correlation was found between phenolic content and the antioxidant assay. It seems that phenolic contents are responsible for free radicle scavenging activity. From the observation, it concluded that methanolic extract rich with polyphenolic content and acetone extract showed the highest amount of tannin content.

Key Words: *Boerhavia diffusa*, Total Phenolic Content, Crude extract, Free radicles, ABTS, DPPH.

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INTRODUCTION

Boerhaavia diffusa Linn.(*B. diffuse*) belonging to family Nyctaginaceae, trailing herb found throughout India and collected after rainy season.[1] In ayurveda, *B. diffuse* used as "rasayana" herb from ancient time possess properties like anti-aging, re-establishing youth, strengthening to cells, enhancing of brainpower, and various disease prevention, it also enhances the resistance of the body against any foreign materials, and improve immune system.[2-4] Various secondary metabolites were found from *B. diffuse*, for example, flavonoid glycosides, isoflavonoids, steroids, alkaloids, phenolic, lignin glycosides, which are responsible for pharmacological properties like diuretic, nephroprotective, free-radicle scavenging, anti-inflammatory, anti-microbial, anti-hepatotoxic, anti-stress, immune booster, hepatoprotective, anti-metastatic and anti-histaminic.[5-7]

Since, last many years, as body knowledge increases, which shows that free radicles are major responsible for many diseases like atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia, degenerative eye disease etc.[8,9] During metabolic process, cell of body produces single electron species called free radicles. There is two types of free radicle species well known such as reactive oxygen species (ROS) and reactive nitrogen species (RNS).[10,11] ROS includes products of lipid peroxidation, protein carbonyl species which lead to the risk of atherosclerosis and protein damaged by protein carbonyl species. RNS includes products of nitric oxide, peroxynitrites which carried out DNA damage, inflammation, proliferation of cancer cells, dysfunction of apoptosis and chronic damage to all biomolecules.[12,13] The medicinal plants showed effective defense mechanisms against the ultraviolet light, other radiation and also abundant sources of various antioxidants. Various pharmacologically active secondary metabolites are available from medicinal plants such as polyphenolic compounds like flavonoids, biflavonols, phenols, etc., and different nitrogen compounds such as alkaloids have been prone to exhibit strong antioxidant activity, among all polyphenols are more intense.[14]

Polyphenols possess two general classes, one is flavonoids and another is phenolic acids. Flavonoids are further partitioned into flavones, flavonols, flavanols, isoflavones and phenolic acids are generally classified into hydroxybenzoic and hydroxycinnamic acids. Dietary polyphenols are potent antioxidants, able to scavenge free radicals and thus preventing damage to cellular molecules.[15] Antioxidant action is not limited to only scavenging of reactive oxygen species (ROS), but also useful in detoxification of cells, modulation of cell signaling and gene expression etc.[16]

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From the literature, concluded that the effect of various extracting solvents and its comparison on total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC) and antioxidant potential in crude extract of root of *B. diffusa* is still paucity.[17,18] So, the aim of present work is evaluation of the solvent extraction process for *B. diffusa* root extract through the determination of its phenolic, flavonoid, tannin content, and antioxidant activity assay.

MATERIAL AND METHOD

Folin–Ciocalteau reagent, DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), Ascorbic acid, Quercetin, Gallic acid were purchased from Sigma–Aldrich. The Solvent used for extraction were analytical grade (Sigma Aldrich) and other chemicals used for various tests were of analytical grade (Sigma Aldrich).

Collection and Identification of plant material

The Root of *B. diffusa* Linn was collected from the local market and authentication was confirmed by NISCAIR/RHMD- Delhi, India, with Ref No. 2018/3279-80-4. The dried root sample was powdered and store in the packed container under the cool condition for further use.

Preparation of plant extracts

Fine root powder of *B. diffusa* (40 gm) was taken in the round bottom flask and extracted with different solvents (200 ml) such as toluene, dichloromethane, chloroform, ethyl acetate, acetone, ethanol, methanol, water, 60% aqueous methanol (60% aq. methanol), 60% aqueous ethanol(60% aq. ethanol), and 60% aqueous acetone(60% aq. acetone) in mechanical stirr at a constant stirring rate (300 rpm) under 25˚C-35˚C. The extraction process repeated for three times an interval of 12 hr. Each solvent extract was filtered through whatman filter paper no. 1 and concentrated under reduced pressure at 45 ˚C -50˚C using rotary evaporator and concentracted extract was further dried under lyophilization. After the lyophilization, dried extract stored under 4˚C -10˚C in airtight container for further use.

Total Phenolic Content (TPC)

The total phenolics content of the dry extracts of *B. diffusa* was determined by Folin-Ciocalteu test, this method slightly modified by Shaikh Abusufyan et al.[19,20] Standard and sample readings were measure by using a spectrophotometer at 765 nm against the blank.

The test sample of *B. diffusa* (0.2 mL) was mixed with 0.6 mL of water and 0.2 mL of Folin-Ciocalteu's (FC) phenol reagent (1:1). After a 5-minute, 1 mL of Na_2CO_3 solution (8% w/v in water) was added to the FC solution and the volume was made up to 3 mL with water and vortexed for 10 minutes. The reaction mixture was kept in the dark place for 30 min and after centrifugation, the absorbance of different samples was measured at 765 nm in a spectrophotometer. Total phenolic content was calculated as GAE/g gallic acid equivalents of dry plant material against on the basis of a standard calibration curve of gallic acid (5-500 mg/L). Overall, all determinations were carried out in triplicate.

Total Flavonoid Content (TFC)

Total flavonoid content was calculated by the aluminum chloride spectroscopic method.[21,22] The 2.5 ml (500 ppm) of the extract was mixed with 2 ml of distilled water and add 2 ml of 5% sodium nitrite, incubated this mixture at 25 ˚C for 5 min. After then add 2 ml of $AlCl_3$ and incubate for 6 min. Add 1 M NaOH solution, mixed vigorously, and kept it for 15 min at room temperature and measured at 512 nm versus blank. The total flavanoid content of different extracts was calculated from the calibration curve, as mg of quercetin equivalent per g dry weight. Calibration of quercetin was prepared in a range of 1- 500 ppm in MeOH. All extracts were analyzed in triplicates.

Total Tannin Content (TTC)

Total tannin content of the dry extracts of *B. diffusa* was determined via polyvinyl polypyrrolidone (PVPP).[23,24]

The test sample of *B. diffusa* (1 ml) (500 ppm), 200 mg PVPP was mixed with 2 ml distilled water and vortexed for 10 minutes. The reaction mass was kept at 40˚C-100˚C for 2 hours and after centrifuging, collect the supernatant and perform the same procedure of Total phenolic content. Supernatant has phenolics constituents, other than the tannins (the tannins would have been precipitated along with the PVPP). From the above results, the Total Tannin Content of the various extracts was calculated as follows:

Tannins (%) = Total Phenolics (%) - Non-Tannin Phenolics (%)

Antioxidant Potential

DPPH Assay

DPPH assay method was used for determining the antioxidant activity. DPPH (α, α-diphenyl-β-picrylhydrazyl, C18H12N5O6, M = 394.33) is stable free radical, which reducing by hydrogen atom from antioxidants and converting to corresponding hydrazine.[25,26]

The % Scavenging effect of Various concentrations of different extracts of *B. diffusa* was measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. The four ml of freshly prepared 0.2mM DPPH solution in methanol and 2 ml of each extract mixed in a range of 50-500 ppm. This solution incubated for 20 min in a dark and cool place. The resulting solution was shaken dynamically for 10 min and incubated for 30 min. The test sample was measured at 517 nm using a spectrophotometer. Here, ascorbic acid was used as a standard. The percentage of DPPH radicle scavenging property of the sample was calculated using the following equation:

$$\%\ \text{radical scavanging activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Ferric Reducing Power Assay

Ferric reducing the capacity of extracts was determined using potassium ferricyanide-ferric chloride method.[27,28] Two ml of different concentration range (50-500ppm) of an extract *B. diffusa*, 2 ml of phosphate buffer (6.6 pH) mixed with 2 ml of 1% Potassium Ferricyanide. Kept this solution at 50°C in a water bath for 20 min. Subsequently, cool the solution at room temperature, added 1.5 ml of aqueous 10% trichloroacetic acid, and 2 ml of 0.1% Ferric Chloride. Measured absorbing power in a spectrophotometer at 725 nm against distilled water. Blank was prepared as an above mentioned method without extract. The Solution of ascorbic acid was used as a positive control. Each extract was assayed in the triplicate manner for each concentration. The concentration of extract corresponding to 50 percent inhibition (IC_{50}) was obtained from the curve of percentage reducing activity against extract concentration.

$$\%\ \text{reducing activity} = \frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of blank}} \times 100$$

ABTS free radicles anion assay

ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) are widely used for the determination of the antioxidant power of natural extract by spectrophotometric analysis based on quenching of stable ABTS radicles.[29,30]

Free radical anion of ABTS was prepared by reacting 10 ml 10mM aqueous solution of ABTS and 10 ml 5 mM potassium persulphate, kept the solution in a dark place at room temperature for 24 hr. Finalized the absorbance of the free radicle of ABTS solution below 1.00±0.02 at 740 nm using the UV Visible spectrophotometer. Incubate the mixture

of 4 ml $ABTS^{\cdot}$ solution and every 2 ml of different extract (50-500nm) at 300C for 6 min and then measured this mixture at 740 nm. Blank was prepared without extract as above procedure. Ascorbic acid used as a positive control. Each sample was assayed in triplicate for each concentration. The extract concentration corresponding to 50 percent inhibition (IC_{50}) was calculated from the curve of inhibition percentage against extract concentration.

$$\% \text{ inhibition activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Statistical analysis

Statistical analysis was carried out with Graph Pad Prism 11 software (Graph Pad Software, Inc., USA), and results are expressed as means ± standard deviation. Differences between means were determined using Tukey Multiple Comparisons. P values <0.05 were regarded as significant. The correlation coefficients (R^2) were calculated to determine their relationship.

RESULT

Impact of solvent on % yield of extracts

In the present study, various solvents were used for extraction of phytoconstituents from *B. diffusa* root powder like toluene, dichloromethane, chloroform, ethyl acetate, acetone, ethanol, methanol, water, an aqueous mixture of methanol, ethanol, and acetone. The Extraction yield of different solvents is shown in Figure 1. The % yield of various extract was given in the following order: 60% aqueous methanol > 60% aqueous acetone > 60% aqueous ethanol > methanol > water > ethanol > acetone > ethyl acetate > dichloromethane > chloroform > toluene >hexane.

Impact of solvent on TPC

The Value of TPC shows in Table 1. The TPC Value ranged from 239.8±0.25 to 9.900±0.51 mg GAE/g and decreases in following order: methanol > acetone > ethanol > 60% aq. methanol > ethyl acetate > chloroform > 60% aq. acetone > 60% aq. ethanol > dichloromethane > water > toluene > hexane. The TPC value of different extracts were calculated from linear regression of gallic acid (y=0.0081X-0.0074, R^2 = 0.9913).

Impact of solvent on TFC

Table 1 shows TFC of different solvent extracts. The TFC value of different extracts were calculated from linear regression of quercetin (y=0.0018X-0.0027, R^2 = 0.9925). The value of flavonoid content was found in following order : methanol > ethanol > acetone > 60% aq. methanol > ethyl acetate > 60% aq. acetone > 60% aq. ethanol > chloroform > dichloromethane > water > toluene > hexane. The range of flavonoid content was 131.1±4.20 to 17.81±2.31 mg QCE/gm.

Impact of solvent on TTC

The amount of TTC of different solvent extracts shown in Table 1 as mg GAE/g. The amount of tannin content was found between 95.06±0.25 to 4.533±0.37. Here, acetone was found significantly higher tannin content compared to other extracts ($p < 0.05$). The lowest tannin content observed in hexane extract ($p < 0.005$).

Impact of solvent on Antioxidant potential

From the observation Table 2, all three antioxidant assay suggested that methanolic extract showed the highest free radical scavenging activity (P<0.005) and hexane extract had the lowest antioxidant potential comparing with other extracts. Statistical similarity, statistical difference, and order of Antioxidant potential of different solvents were found similar in ABTS and DPPH *in vitro* assay model. But in FRAP assay 60 % aq. ethanol and dichloromethane are statistically similar, which was not found in data of DPPH and ABTS. So there is always a need to perform more than two assay models if possible, because every model has different sensitivity against free radicles.

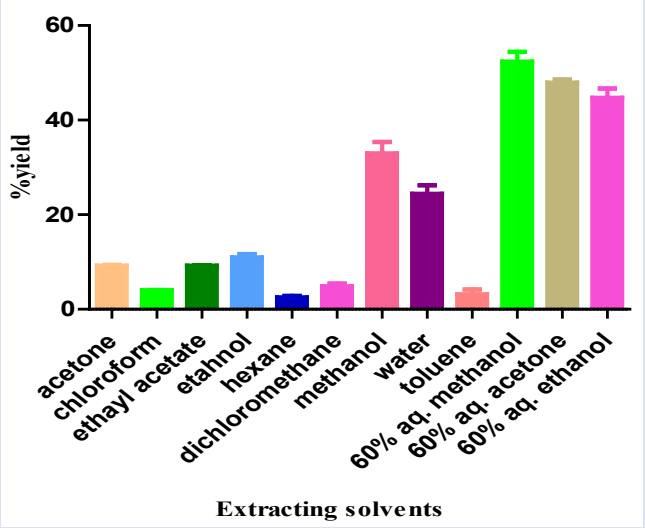

Figure 1: Effect of varying extracting solvents on extract yield of *B.diffusa* with Mean ± SD.

Table 1: Value of TPC, TFC and TTC of different extract of *B. diffusa*.

Solvents	$TPC_{(mg\ GAE/g)}$	$TFC_{(mg\ QCE/g)}$	$TTC_{(mg\ GAE/g)}$
acetone	151.4 ± 0.79^{b}	120.0 ± 1.70^{cb}	95.06 ± 0.25^{a}
chloroform	87.42 ± 0.62^{f}	64.11 ± 20.21^{h}	47.08 ± 0.51^{j}
ethyl acetate	98.86 ± 0.49^{e}	89.67 ± 1.11^{e}	59.01 ± 0.43^{g}
ethanol	146.5 ± 0.49^{c}	125.6 ± 2.31^{ba}	86.42 ± 0.25^{c}
hexane	9.900 ± 0.51^{l}	17.81 ± 2.31^{l}	4.533 ± 0.37^{l}
dichloromethane	84.05 ± 0.49^{ih}	54.11 ± 1.11^{ih}	47.00 ± 0.75^{kj}
methanol	239.8 ± 0.25^{a}	131.1 ± 4.20^{a}	90.78 ± 0.87^{b}
toluene	64.63 ± 0.38^{k}	31.52 ± 1.69^{klj}	51.11 ± 0.43^{f}
water	74.09 ± 0.38^{j}	41.52 ± 0.6^{ji}	65.35 ± 0.28^{ed}
60% aq. methanol	117.1 ± 0.24^{d}	90.04 ± 0.64^{de}	65.43 ± 0.49^{d}
60% aq. acetone	86.35 ± 0.38^{gf}	71.52 ± 0.64^{fhig}	48.07 ± 0.51^{ijkh}
60% aq. ethanol	85.28 ± 0.25^{hg}	67.44 ± 1.11^{ghi}	48.31 ± 0.57^{hjk}

Values are mean ± SD of three replicate determinations (n=3) ± standard deviation. Mean values followed by different superscripts (a-l) in each column are significantly different (P< 0.05).

Table 2: IC50 (µg/ml) value of DPPH free radicle assay, FRAP, $ABTS^{\cdot-}$ scavenging.

Solvents	DPPH	FRAP	ABTS
acetone	3.49 ± 0.11^{b}	3.85 ± 0.38^{b}	4.63 ± 0.70^{b}
chloroform	8.85 ± 1.13^{fc}	8.81 ± 0.56^{fc}	10.75 ± 1.30^{fc}
ethyl acetate	7.47 ± 0.66^{c}	8.39 ± 0.33^{c}	8.179 ± 0.43^{c}
ethanol	3.40 ± 0.32^{b}	3.81 ± 0.22^{b}	3.99 ± 0.38^{b}
hexane	33.59 ± 1.27^{k}	34.21 ± 2.09^{k}	36.43 ± 0.59^{k}
dichloromethane	10.63 ± 0.07^{hf}	11.04 ± 0.17^{hf}	12.62 ± 0.94^{hf}
methanol	0.94 ± 0.03^{a}	1.07 ± 0.05138a	1.20 ± 0.1000a
toluene	20.14 ± 0.37^{j}	19.73 ± 0.66^{j}	24.55 ± 2.59^{j}
water	16.77 ± 1.09^{i}	16.19 ± 1.52^{i}	18.37 ± 0.87^{i}
60%aq. methanol	7.90 ± 0.9467^{dfce}	7.55 ± 0.56^{dfce}	8.23 ± 1.44^{dfce}
60% aq. acetone	10.30 ± 0.17^{gfh}	11.15 ± 0.13^{gfh}	11.76 ± 0.35^{gfh}
60 % aq. ethanol	8.360 ± 0.59^{efcg}	9.035 ± 0.22^{efcgh}	8.932 ± 0.09^{efcg}
Ascorbic acid	0.012 ± 0.01	0.091 ± 0.12	0.021 ± 0.12

Values are mean ± SD of three replicate determinations (n =3) ± standard deviation. Mean values followed by different superscripts (a-l) in each column are significantly different (P< 0.05).

DISCUSSION

The result of this work showed that different solvents had a significant effect on the extraction yield of *B. diffusa* root. The highest yield was found in 60% aqueous methanol, followed by 60 % aq. acetone and 60% aq. ethanol. The toluene and hexane extracts were found lower extraction yield. Observation showed aqueous solvent was extracted more solid as compared to other solvents. So, a mixture of water and organic solvent might be facilitating the extraction of phytoconstituents soluble in water as well as organic solvents. These findings were in agreement with previous studies on *L. aromatica*[31], *C. calcitrans* [32] and *S. chinensis*.[33]

Folin-Ciocalteu reagent is used for testing of TPC in the extract, electron rich hydroxyl groups interact with specific redox reagents (Folin-Ciocalteu reagent) to form a blue chromophore constituted by a phosphotungsticphosphomolybdenum complex and subsequently it quantified by UV visible spectrometer. The intensity of the blue chromophore complex depends on the concentration of the phenolic compounds in the extract[34,35]. The TPC value of chloroform and 60 % aq. acetone are statistically similar (P > 0.0001). There were also statistical similarity between dichloromethane - 60% aq. ethanol and 60 % aq. ethanol - 60% aq. acetone. From the above result concluded that solvent with higher polarity like methanol, acetone, and ethanol are effective for the extraction of TPC from *B. diffusa*. As per Apurba Sarker Apu *et al* methanol extract of aerial part of B. *diffusa* Linn showed significant phenolic content as compared to that hexane and ethyl acetate extracts, which is correlated with our result of TPC.[36] This result of TPC is similar to the result of Taha M. Rababah *et al*.[37], Kandhasamy Sowndhararajan *et al*.[38] and Su Chern Foo *et al*.[32] Number of phenolic compounds are isolated from *B. diffusa* root extract. A solvent with lower viscosity can easily penetrate to pores of the cell wall of plant material to leach out bioactive phytochemicals than solvent with high viscosity as well as the polarity of solvents also affected.[39,40] So, the complexity and nature of the phenolic structure, viscosity, and polarity of the solvent may affect TPC of plant material.[41,42] In our study methanol is the best solvent.

For determination of TFC, aluminum chloride test is used, it gives color complex with a phenolic hydroxyl group, and measured by UV visible spectrometer.[43] The result of TFC was similar with study of *O. parvifolia* by Rajan Murugan.[44] Flavonoids are more common polyphenol found in nature. Free radical species such as superoxide ($O_2^{\bullet-}$), the hydroxyl radical ($^{\bullet}OH$) and the lipid peroxyl radical ($LOO^{\bullet}$) scavenge by flavonoids as donating an electron or hydrogen atom and the phenolic hydroxyl group responsible for scavenging these radicals.[45,46] Various flavonoids are isolated from *B. diffusa* root and literature reveals that it shows scavenging potential too.[47,48] Several solvents, such as ethanol, acetone, chloroform ethyl acetate, methanol, acetone and their aqueous combinations have been used for the extraction of polyphenolic from plants matrices, often with different proportions of water.[49]

Tannins are one of the type of polyphenolic compounds with high molecular weight. All phenolic constituents including tannin, flavonoid are covered in the test of Folin Ciocaltue reagent. For the determination of total tannin content, extract treated with PVPP which irreversibly bind with tannin-phenolic and remaining phenolic measured by Folin Ciocaltue reagent. This result is related to the study of bunga kantan inflorescence.[50] Comparison between TPC, TFC and TTC are similar with the result of different extracts of *Bauhinia vahlii*[51] and *Osbeckia parvifolia*.[52]

DPPH is synthetic free radicle with three benzene rings with an unpaired electron of centered nitrogen. Due to this odd electron, the alcoholic solution of DPPH solution shows maximum UV absorption at 517 nm in the UV visible spectrophotometer. Antioxidants with reducing potential react with an unpaired electron of DPPH and color of reduced DPPH will be changed from purple to yellow, which shows lower absorption at 517 nm.[53]

Potassium ferricyanide (Fe^{3+}) reduced in the presence of substance with the antioxidant potential to form potassium ferrocyanide (Fe^{2+}) at pH 6.6 (yellow in color). Ferric chloride reacts with ferrocyanide and to form Prussian blue-green water soluble ferric ferrous complex, has lambda max at 700 nm.[54]

The reaction of potassium per-sulfate with ABTS (colorless solution) is generated the Stable free radicle of ABTS. The Blue-green color of the radical anion (ABTS•–) absorbs 734 nm in UV visible spectrophotometer. Falling in absorbance of radical anion of ABTS shows the antioxidant potential of phytoconstituents.[55] Phtytocontituents with antioxidant potential that inhibit oxidation by reducing or quenching free radicles. Free radical scavenging constituent acts via various mechanisms as hydrogen atom transfer, single electron transfer, and chelation of transition metal. Thus, the process of damaging of normal cells by free radicles will be suppressed by antioxidant phytoconstituents. Constituents with -OH, $-NH_2$, -SH group in aromatic ring responsible for antioxidant power of particular constituents. Generally, polyphenolic compounds contain phenolic hydroxyl groups that can provide a hydrogen atom or an electron to free radical, and unpaired electrons of phenolic compounds are delocalized in the extended conjugated aromatic system.[56] Various models are available for assay of the antioxidant power of different chemicals like DPPH, FRAP, ABTS, ORAC, etc.[55] In the present work, three different models were used for the *in vitro* assay of phytoconstituents. The Free radical scavenging ability of different extracts was compared based

Table 3: Correlation coefficient (R^2) between TPC, TFC, TTC, and Antioxidant assay.

	TPC	TFC	TTC	DPPH	FRAP	ABTS
TPC		0.977	0.868	0.897	0.9	0.900
TFC	0.977		0.814	0.888	0.889	0.894
TTC	0.868	0.814		0.610	0.612	0.609
DPPH	0.897	0.888	0.610		1.000	0.999
FRAP	0.900	0.889	0.612	1.000		0.999
ABTS	0.900	0.894	0.609	0.999	0.999	

on the IC_{50} value of different extracts. The IC_{50} indicated how much concentration needed to inhibit response at 50 % level. The lower value of IC_{50} indicates good scavenging property of free radicles by phytoconstituents.

The Methanolic extract showed significantly the highest antioxidant ability ($p<0.05$). The lowest reducing power was found in hexane and toluene extract as compared to other extracts. From the study, it concluded that methanol, acetone, ethanol solvents are more favorable for isolation polyphenolic constituents from extract of *B. diffusa.*

Correlation between TPC, TFC, TTC and Antioxidant assay

The experimental data of different antioxidant assay, TPC, TTC and TFC were compared and correlated with each other and its correlation coefficient (R^2) is shown in Table 3. The Total phenolic content shows 90.0 %, 89.7% and 90.0 % correlation for ABTS, DPPH, and FRAP respectively. Similarly flavonoids contribute to 89.4 % (ABTS), 88.9 % (DPPH), 88.9 % (FRAP) for antioxidant activity. The total tannin content contributes only 61.0 % (DPPH), 61.2 % (FRAP), 60.9 % (ABTS) for the antioxidant property. TPC, TTC, and TFC also showed a good correlation with each other and are ranged from 87% to 98%. This concluded that polyphenolic constituents are responsible for major antioxidant activity and the remaining part of antioxidant activity comes from non-phenolic contents like vitamins, carotenoids, etc.[57] The good correlation observed between all three antioxidant models, which means the sensitivity of all three *in vitro* assay are good.

CONCLUSION

The present work revealed that different types of solvents have a major influence on phenolic content and antioxidant activity. In general, extraction yield increases with an increased amount of water content with organic solvents. In contrast, the highest level of antioxidant is observed in the methanolic extract of *B. diffusa,* when compared with other solvents. This result indicates that methanolic extract of *B. diffusa* could serve as potential herbal medicine against free radical-induced oxidative damage. It provides the basis for food additives and enhances nutritive value.

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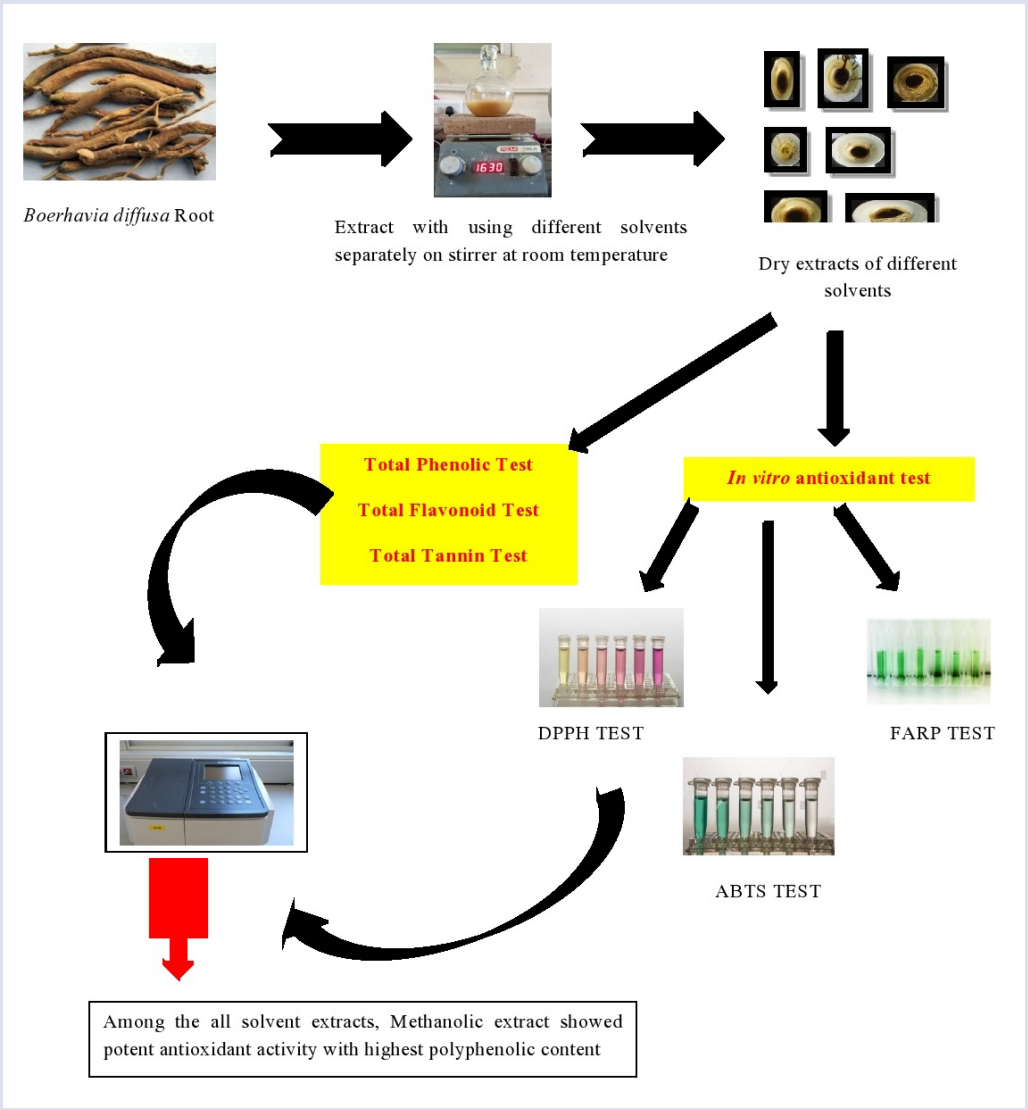
GRAPHICAL ABSTRACT



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