ISSN (Online): 2455-7838
SJIF Impact Factor (2016): 4.144


EPRA International Journal of

# Research & Development

## (IJRD)


**Monthly Peer Reviewed & Indexed International Online Journal**

**Volume:1, Issue:10,December 2016**

Published By : EPRA Journals

SJIF Impact Factor: 4.144 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 1 | Issue : 10 | December | 2016

# PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY INVESTIGATIONS ON THE CRUDE EXTRACTS OF BRUCEA ANTIDYSENTERICA LEAVES

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## ABSTRACT

*The aim of this study was to screen various solvent extracts of Brucea antidysenterica leaf to display potent antioxidant activity in vitro, total phenolic and flavonoid contents in order to find possible sources for future novel antioxidant activity in pharmaceutical formulations. Phytochemical screening showed the presence of alkaloids, flavonoids, phenols, Saponins, triterpenoids and steroids in large amounts in 80% methanol crude extract. The results revealed that the aqueous soluble fraction exhibited the highest percent inhibition (87.57 ± 1.15 %) of the DPPH radical as compared to the other fractions and crude extracts. It also showed the highest FRAP value (72.40 ± 0.22 mg AAE/g of fraction). The various crude extracts and fractions of Brucea antidysntrica significantly had antioxidant power and reduced the DPPH. Leaf of Brucea antidysenterica can be used as source of natural antioxidant and a potential alternative medicine for oxidative stress related to non-communicable chronic diseases associated with free radical and antibacterial activity.*

**KEYWORDS**: *antioxidant activity;Brucea antidysntrica; DPPH assay; phenolics*

## INTRODUCTION

Natural products are important sources for biologically active drugs. There has been an increasing interest in the medicinal plants as natural products in different parts of the world.[1] The medicinal value of these plants depends on bioactive phytochemical constituent's action in the human body. Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components.[2]

Herbs are used in many domains, including medicine, nutrition, flavouring, beverages, dyeing, repellents, fragrances, cosmetics.[3] Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, antiinflammatory, antimicrobial, hypolipidemic, antimutagenic effects and anticarcinogenic potential.[4]

Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer. Crude extracts of herbs and spices, and other plant materials rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food.[5] For this purpose, extracts of different plants have been tried with success as reducing agents. In this study, the reducing agent comes from extracts of *Brucea antidysenterica*, which is a plant rich in alkaloids, quassinoids and polyphenols. These molecules are potentially strong reducing agents due to their numerous OH groups that promote their antioxidant activity.[6]

This investigation, therefore, attempt to estimate secondary metabolites and evaluate the antioxidant activity of the leave of *Brucea antidysentrica.*

## GENERAL OBJECTIVE

The main objective of this study is to assess the phytochemical screening and antioxidant activities of crude extracts from the leave of species *Brucea antidysenterica* using different solvents.

## SPECIFIC OBJECTIVES

The specific objectives of this study are:

- ✧ To assess the secondary metabolites from the crude extracts and its fractions of *Brucea Antidysenterica* leaves.
- ✧ To determine the total phenolic contents of the crude extracts and its fractions.
- ✧ To determine the total flavonoid contents of the crude extracts and its fractions
- ✧ To evaluate the antioxidant effects of crude extracts and its fractions of *Brucea antidysenterica* leaves using different solvents.

## MATERIALS AND METHODS

### Sample preparation

#### a) Crude extract preparation

The collected fresh leaf of *Brucea antidysenterica* was cut into small bits to facilitate drying and air dried at room temperature for three weeks under a shed until it became well dried. The dry plant materials, leaf take separately and grind to a uniformly using an electric grinder to obtain a fine powder.[7] The ground plant material in the extracting solvent was placed on ice in a water bath for 1 h.

The powdered leaf (10 g) was homogenization with absolute methanol (100 mL), 80% methanol (100 mL), chloroform (100 mL), petroleum ether (100 mL) placed on a shaker and soaked for 48 hours. The residue was filtered using a Buchner funnel under vacuum through Whatman No. 1 filter paper. Chlorophyll and water were removed using activated charcoal, and $MgSO_4$ respectively and concentrated using Böchi rotary vacuum evaporator under reduced pressure at 40 $^{o}$C temperature to obtain the crude extract.

#### b) Fractionation of crude extract

Solvent-solvent partitioning was done using the protocol designed.[8] Given that the sample containing 80% methanol extract of leaf was the best extract amongst all the crude extracts and it was chosen for further fractionation.

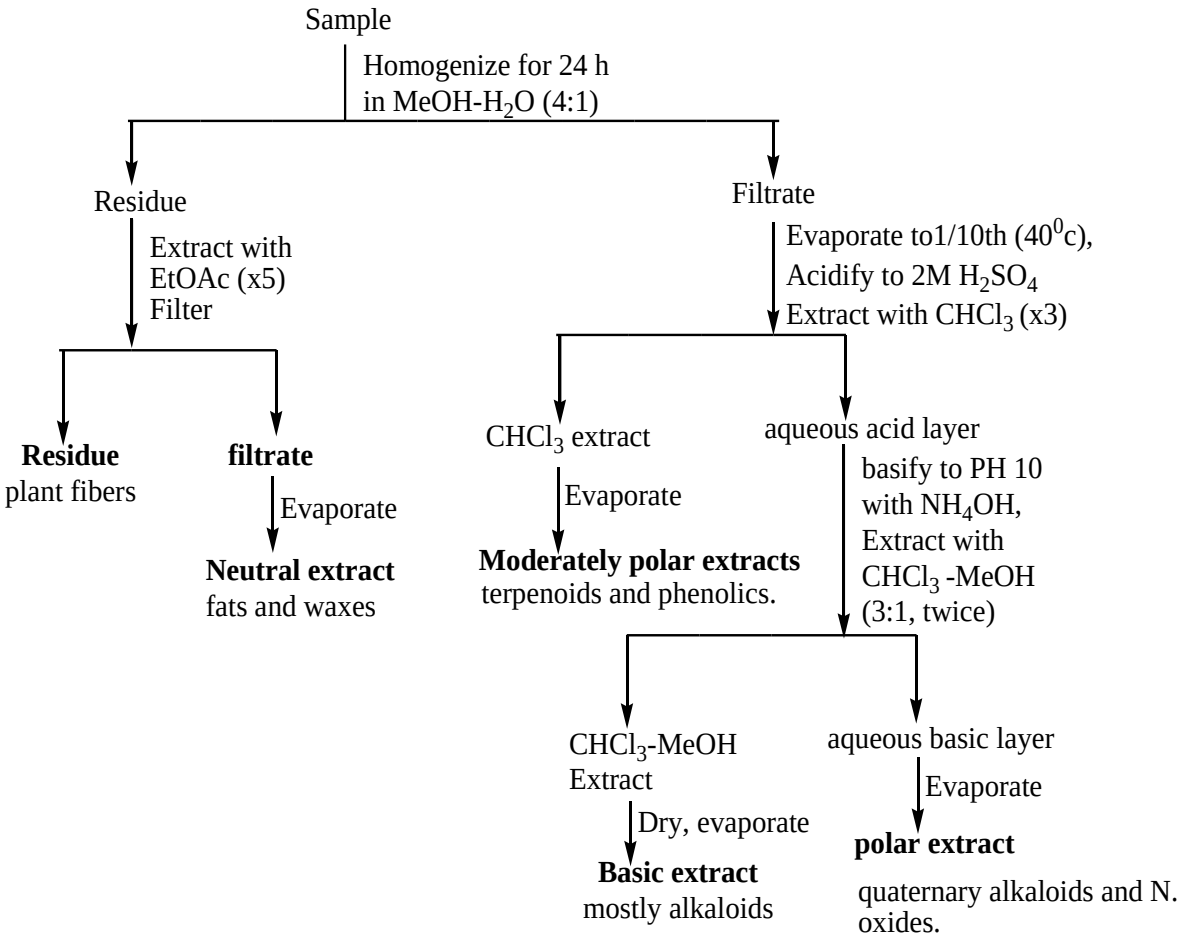


**Figure 1: A general procedure for extracting and fractionating plant sample.**

## EXPERIMENTAL PROCEDURES

### Procedure for phytochemical screening

Phytochemical screening of the crude absolute methanol, 80% Methanol, chloroform, peterolum ether and solvent fractions of 80% methanol extract of *Brucea antidysenterica* was phytochemically screened using different chemical assays to identify the presence or absence of the phytochemical components in the plant extracts[8,9,10,11]

### Determination of total phenolic content

The total soluble phenols present in different solvent extracts and fractions of crude extract of leaf were determined by using the Folin-Ciocalteu reagent according to the procedure reported.[12]

### Determination of total flavonoid content

The aluminum chloride colorimetric assay was used for total flavonoid determination, as described.[13]

### Determination of reducing power

Reducing power assay was determined according to the method.[14]

### Determination of DPPH˙ radical scavenging activity

The antioxidant activities of the extract were evaluated Sphectrophotometrically following DPPH method with little modification.[15]

### Statistical analysis

All experiments were carried out in triplicate. One-way analysis of variance (ANOVA) was used to analyze the data using Orgine 6.0 software to test any significant differences among various treatments. Correlations between variables were established by regression analysis.

## RESULTS AND DISCUSSION

### Yield of extraction

The four extracting solvents, absolute methanol, 80% methanol, Chloroform, Petroleum ether and fractions of 80% methanol solvent extract were evaluated for their effectiveness to extract bioactive compound from the leaf of *Brucea antidysntrica* that had been air-dried. The 80% methanol solvent was superior in their ability to extract the metabolites and 80% methanol extract

(17.2 g/100 g dry weight) was significantly more efficient than absolute methanol (8.4 g/100 g dry weight) towards extracting more metabolites. Extraction with petroleum ether offered the least yield (3.2 g/100 g dry weight) of the crude extracts. The aqueous fraction of *Brucea antidysntrica* was high in quantity (7.5 g/100 g dry weight) among the other solvent fractions of 80% methanol crude extract whereas the yield of chloroform soluble fraction was the lowest (1.2 g/100 g dry weight) (Table 1).

Based on the present study, it could be suggested that aqueous based organic solvents are superior to recovering a higher extraction yield of components from *Brucea antidysntrica* and thus might be considered in future extraction studies.

However, it is important to point out that optimal extraction yield may not translate to higher antioxidant activity; the aqueous based solvents may just solubilise a larger range of compounds, some of which may have little or no antioxidant activity.

**Table 1: Effect of extracting solvent on the extraction yield ( g/100 g dry weight ) from *Brucea antidysenterica.***

| Extracting solvent | Extraction yield (g/100 g dry weight) |
|---|---|
| **Crude extract** | |
| Absolute methanol | 8.4 ± 0.12 |
| 80% methanol | 17.2 ± 0.24 |
| Chloroform | 4.8 ± 0.03 |
| Peterolum ether | 3.2 ± 0.04 |
| **Fractionations of 80%methanol crude extract** | |
| Chloroform fraction | 1.2 ± 0.11 |
| Chloroform-methanol fraction | 1.8 ± 0.23 |
| Aqueous fraction | 7.5 ± 0.03 |

## Qualitative phytochemical analysis

The preliminary phytochemical analysis of different solvents of crude extracts and fractions of *Brucea antidysntrica* is depicted in Table 2 below.

**Table 2: Results of phytochemical screening of the various crude extracts and fractions of *Brucea antidystrica.***

| Test | AME | 80% ME | CE | PE | Fr-3 | Fr-2 | Fr-1 |
|---|---|---|---|---|---|---|---|
| Alkaloids | + | ++ | - | - | + | +++ | ++ |
| Terpenoids and Steroids | + | + | +++ | ++ | +++ | ++ | + |
| Saponins | ++ | +++ | - | - | - | + | +++ |
| Tannins | - | - | - | - | - | - | - |
| Phenolics | + | +++ | - | - | + | ++ | +++ |
| Flavonoids | ++ | +++ | - | - | ++ | ++ | +++ |
| Glycosides | + | ++ | - | - | - | + | +++ |

*Phytochemical costituents of the crude extracts and fractions of Brucea antidystrica ((+)-Test Positive, (++)-present in relatively moderate larger amount, (+++)-relatively larger amount,( - )-Test Negative). AME=absolute methanol, 80% ME=80% methanol extract, CE= chloroform extract, PE=petroleum ether extract, Fr-1 = Aqueous fraction, Fr-2 = chloroform-methanol fraction, Fr-3 =chloroform fraction.*

The results of *Brucea antidysntrica* leaf extracts revealed that the presence of terpenoids and steroids in all extracts studied. Glycosides were detected in absolute methanol, 80% methanol, chloroform-methanol fraction and aqueous soluble fraction but absent in chloroform fraction, chloroform extract and peterolum ether extract. Aqueous fraction, 80% methanol extract, and absolute methanol extract contain triterpenoids and steroids with very lesser degree of precipitation (Table 2).

Phenols, triterpenoids, and steroids showed moderate degree of precipitation (++) in the chloroform-methanol fraction whereas alkaloids show

higher degree of precipitation (+++). The higher degree of precipitation (+++) of triterpenoids, and steroids were observed in chloroform fraction and chloroform extract.

In the 80% methanol extract, flavonoids, phenols, and saponin showed higher amount of precipitation (+++) whereas alkaloids and glycosides showed very moderate degree of precipitation (++). The results showed that the plants are rich in alkaloids, flavonoids, phenols, saponins, triterpenoids and steroids. The presence of these substances in the investigated plant accounts for their usefulness as medicinal plant. The degree of precipitation of secondary metabolites varies with solvents. This may be due to various degrees of solubility of different solvents for different phytoconstituents. These tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. It would thus mean that in this study, the hydro-alcohol extracts had the highest number of bioactive compounds. Since the yield of bioactive metabolites in a plant extract also varies considerably with the solvent of extraction it is plausible that the hydro-alcohol extracts were generally more potent than the organic solvent probably because the active compounds in the plant dissolved more readily in and were better extracted by a more hydro-alcoholic (80% methanol extract) solvent.

The preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may locate the source of pharmacologically active chemical compound.

The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proven to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

## Total phenolic content (TPC)

The results of the total phenolic content determination of the examined plant extracts and fractions was presented (**Table 4**). The content of total phenols in different solvent extracts and fractions was expressed as gallic acid equivalents (GAE) per gram of dry extract/fraction. To calculate the total phenolic content, calibration curve (absorbance *vs* concentration at 750 nm) of gallic acid was constructed (Figure 2). The content of total polyphenol in the *Brucea antidysntrica* sample were drived from figure 2 ($y = 0.0014x + 0.04399$, $R^2 = 0.998$).

**Table 3: Absorbance of standard compound (gallic acid)**

| Concentration (µg/ml) | Absorbance |
|---|---|
| 10 | 0.056 ± 0.04 |
| 60 | 0.102 ± 0.01 |
| 120 | 0.171 ± 0.04 |
| 180 | 0.233 ± 0.02 |
| 240 | 0.294 ± 0.01 |
| 300 | 0.355 ± 0.01 |

Values expressed are mean ± standard deviation of three measurements.

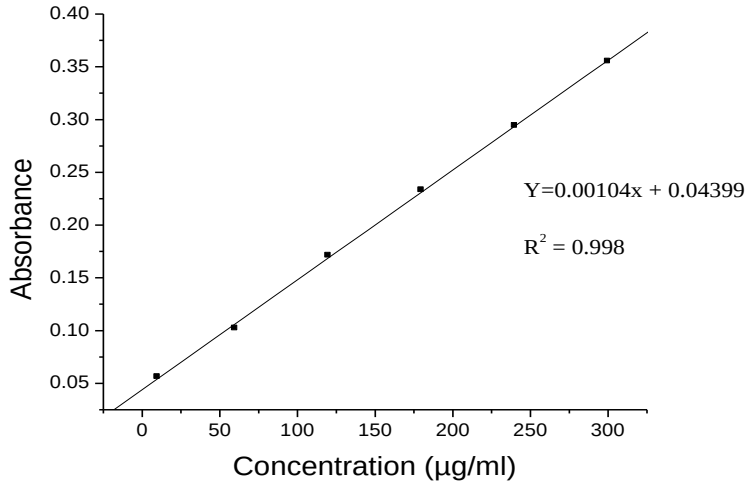


**Figure 2: Calibration curve of standard gallic acid for determination of total phenolics content.**

The phenolic compounds in plant extract are more often associated with other molecules like proteins, polysaccharides, terpenes, chlorophyll and inorganic compounds. Hence, it requires suitable solvent for extraction. The phenolic extracts and fractions of plants are always a mixture of different classes of phenols, which are selectively soluble in the solvents. The use of an alcoholic solution provides satisfactory results for the extraction process.[16]Aqueous alcohol (80%methanol extract) solvents are the best solvents for extraction of phenolic compounds from *Brucea antidysntrica* leaves. Petroleum ether is inefficient solvent for extraction of total phenols from *Brucea antidysntrica*. The average total phenolic content (mg GAE/g crude extract) of 80% methanol extract was significantly high (71.53 ± 0.04 mg/g) than absolute methanol, chloroform, and petroleum ether extract (38.87 ± 0.02 mg/g, 37.53 ± 0.02 mg/g, and 12.78± 0.01 mg/g, respectively). The use of mixture alcohol and water present the advantage of modulating the polarity of alcohol solvents, also adding that solubility of polyphenols depends mainly on the hydroxyl groups, the molecular size and the length of hydrocarbon. These mixtures become ideal and selective to extract a great number of bioactive compounds of which phenolic compounds.

Total phenolic content of the different fractions of *Brucea antidysntrica* was solvent dependent and expressed as milligrams of gallic acid equivalents (GAE) per g of extract. The total phenolic content in aqueous fraction was highest at 148.21±0.03 mg of GAE/g of sample, and the yield also the highest among the fractionated extracts (**Table 4**). The aqueous fraction, chloroform-methanol fraction, and chloroform fraction had different amount of phenolic content, which were 148.21±0.03, 104.25 ± 0.02 and 61.38±0.05 mg of GAE/g of extract, respectively. The high phenolic content of aqueous soluble fraction may have contributed towards its antioxidant and related activity.

**Table 4: Total phenolics content of crude extracts and soluble fractions from leaf of *Brucea antidysntrica*.**

| Plant<br>Crude extracts | Total phenolics<br>(mg Gallic Acid equivalent/g) |
|---|---|
| 80% methanol extract | 71.53 ± 0.04 |
| Absolute methanol extract | 38.87 ± 0.02 |
| Chloroform extract | 37.53 ± 0.02 |
| Petroleum ether extract | 12.78 ± 0.01 |
| **Fractionations of 80% methanol crude extract** | |
| Chloroform fraction | 61.38 ± 0.05 |
| Chloroform-methanol fraction | 104.25 ± 0.02 |
| Aqueous fraction | 148.21 ± 0.03 |

In all case, total polyphenol contents using different solvents and fractions showed in a similar decreasing order: Aqueous fraction > chloroform-methanol fraction >80%methanol extract > chloroform fraction > Absolute methanol extract > chloroform extract > petroleum ether extract. The high concentration of polyphenolics in the aqueous fraction may be due to purification and concentration of phenolics throughout the fractionation procedure and it is probably responsible for its high free radical scavenging activity. The Folin–Ciocalteu's phenol reagent reducing capacity of different fractions is due to presence of hydroxyl groups present in the polyphenolics and flavonoids.

The key role of phenolic compounds as scavengers for free radicals is emphasized in some previously published reports. It was reported that presence of hydroxyl groups contribute directly to antioxidant effect of the system and it also has an important role in preventing lipid oxidation.[17]

Many researchers reported influence of different extraction solvents, techniques on the content of natural antioxidants in extracts. Efficiency of solvents and methods are strongly dependent on plant matrix used.[18] Solvents, such as methanol, ethanol, acetone, propanol and ethyl acetate have been commonly used for the extraction of phenolics from fresh produc.[10] The properties of extracting solvents significantly affected the measured total phenolics content and antioxidant capacity in fruits and vegetables. Very important parameter is solvent polarity – higher the polarity, better the solubility of phenolic compounds. The highest extract yields were obtained with polar alcohol based solvents.[19]

## Total falvanoid content (TFC)

The maximum phenolic content was found in the 80% methanol extract (71.53 ± 0.04 mg /g) of crude extracts of *Brucea antidysntrica* leaf. The amount of total falvanoids was determined with the

quercetin standard compound and the total falvanoid were expressed as mg QE/g of sample.

Quercetin equivalent using the standard curve equation: y= 0.10905 x + 0.0103, $R^2$ = 0.999, Where y is absorbance at 510 nm and x is total flavonoid content in the different solvent extracts, and fractions expressed in mg/mL.

**Table 5: Absorbance of standard compound (quercetin)**

| Concentration (µg/ml) | Absorbance |
|---|---|
| 2 | 0.224 ± 0.02 |
| 4 | 0.438 ± 0.01 |
| 6 | 0.649 ± 0.01 |
| 8 | 0.850 ± 0.03 |
| 10 | 1.105 ± 0.02 |

*Values expressed are mean ± standard deviation of three measurements*

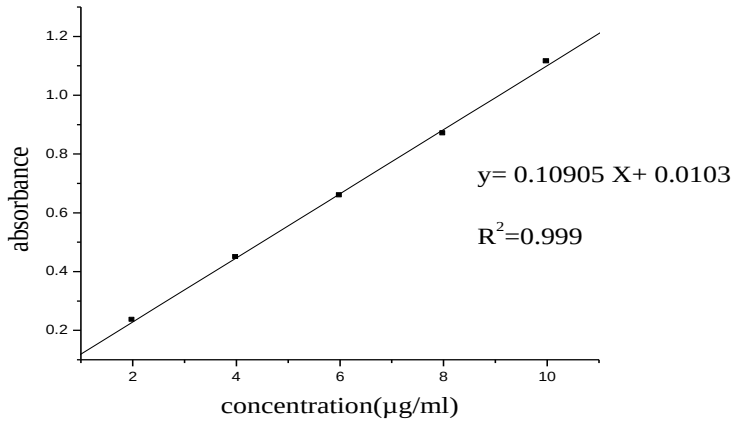


**Figure 3: Calibration curve of standard quercetin for determination of total falvonoid content.**

Table 6 shows the contents of total falvonoid that was measured by $AlCl_3$ reagent in terms of quercetin acid equivalent. The total flavonoid varied from 76.53 ± 0.03 to 1.62 ± 0.02 mg/g, 116.43 ± 0.02 to 46.84 ± 0.05mg/g in the crude extracts, and fractions, respectively. As shown in table 6, the results obtained from this study that of the crude extracts and fractions, the maximum flavonoids content was found in the 80% methanol (76.53 ± 0.03 mg/g) and its aqueous fraction (116.43 ± 0.02 mg/g) of *Brucea antidysntrica* leaf, The aqueous fractions and extracts have the highest level of flavonoids (Table 6). This can be attributed to the fact that aqueous fraction selectively removed and concentrated more a polar compounds from *Brucea antidysntrica*.[20]

**Table 6: Total flavonoid content of crude extracts and 80% methanol fractionations (mg quercetin equivalent/g)**

| Plant<br>Crude extracts | Total flavonoid<br>(mg quercetin equivalent/g) |
|---|---|
| 80% methanol extract | 76.53 ± 0.03 |
| Absolute methanol extract | 21.35 ± 0.05 |
| Chloroform extract | 18.10 ± 0.01 |
| Petroleum ether extract | 1.62 ± 0.02 |
| **Fractionations of 80% methanol crude extract** | |
| Aqueous fraction | 116.43 ± 0.02 |
| Chloroform-methanol fraction | 49.47 ± 0.02 |
| Chloroform fraction | 46.84 ± 0.05 |

This can be due to variation in affinity of the extraction solvents for *Brucea antidysntrica* leaves constituents in terms of their different extraction conditions such as polarity of extracting solvents and temperature.[21] Which exhibited the greatest antioxidant activity thus can be used to explore new drugs. Total flavonoids contents using different solvents and fractions are showed in the following order: aqueous fraction > 80% methanol > chloroform-methanol fraction > chloroform fraction > absolute methanol > chloroform > and petroleum ether extract.

Plants rich in flavonoids are potential sources of natural antioxidants that would add to the overall antioxidant capacity of an organism and inhibit lipid peroxidation. Therefore, the result suggested that phenolic acids and flavonoids may be the major contributors for the antioxidative properties and inhibitory actions toward the oxidative reaction in *vitro* and *in vivo*.[22]

## Reducing rower (FRAP)

Aqueous solutions of ascorbic acid standard at different concentrations (8, 16, 24, 32, 40, 48 µg/mL) were used to construct the calibration curve and the absorbance due to the reducing power of ascorbic acid was recorded. A graph of absorbance *versus* construction was drawn in which a straight line with an equation, y= 0.01579x- 0.08767 and a linear regression coefficient ($R^2$) of 0.998 was obtained (Figure 4).

Many studies have indicated that antioxidant effect is related to the development of reductones were reported to be terminators of free radical chain reactions.[23] Thus, the antioxidant activity of *Brucea antidysntrica* extracts/fractions may relate to their reducing ability.

**Table 7: Absorbance of standard compound (ascorbic acid)**

| Concentration (µg/ml) | Absorbance |
|---|---|
| 8 | 0.052 ± 0.06 |
| 16 | 0.161 ± 0.10 |
| 24 | 0.277 ± 0.04 |
| 32 | 0.430 ± 0.27 |
| 40 | 0.549 ± 0.22 |
| 48 | 0.682 ± 0.15 |

*Values expressed are mean ± standard deviation of three measurements*

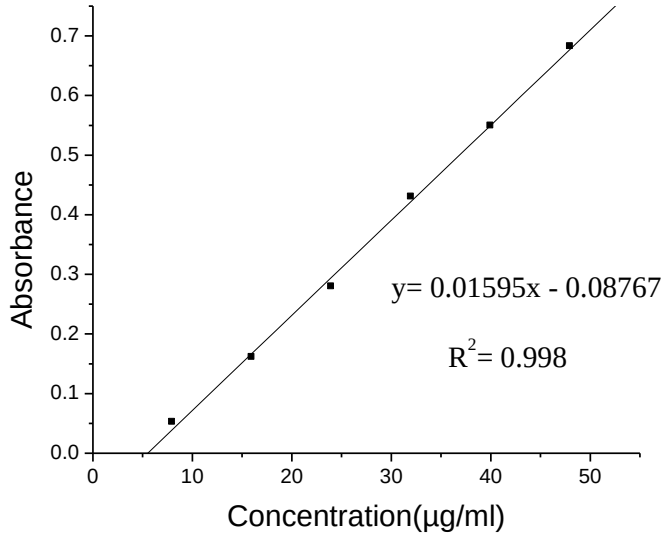


**Figure 4:** Calibration curve of standard ascorbic acid for determination of total antioxidant capacity.

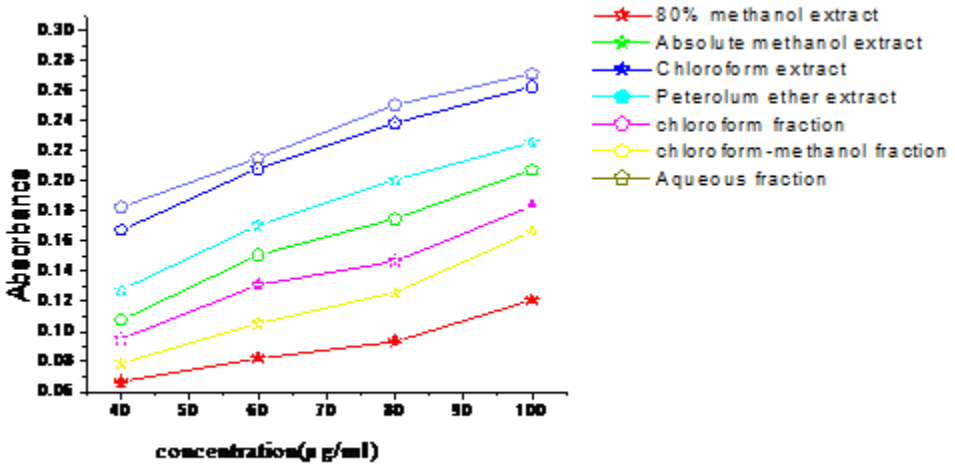


**Figure 13: Reducing power of the samples extract/fractions by $Fe^{3+}$ to $Fe^{2+}$**

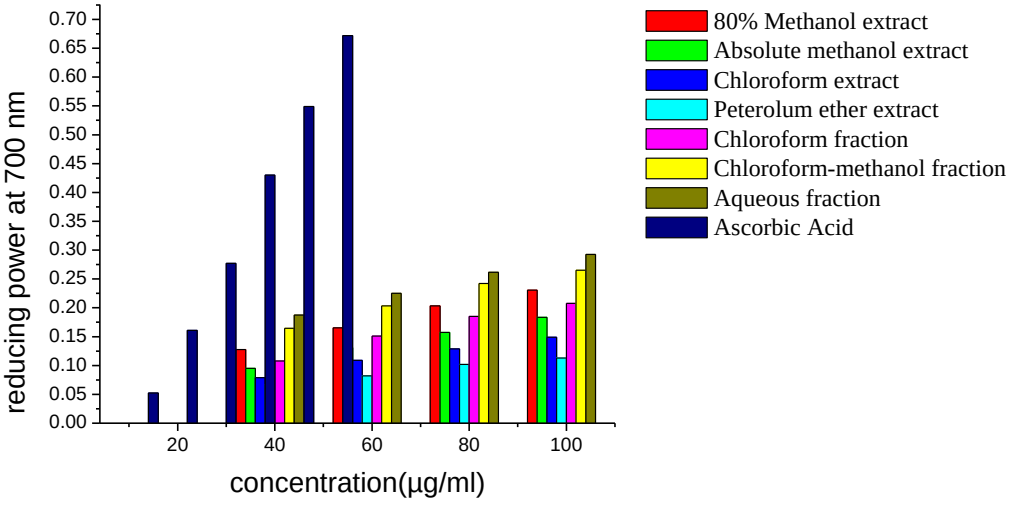


**Figure 5**: The reducing power of *Brucea antidysntrica* extracts/fractions and positive reference standards (ascorbic acid).

The reducing power of *Brucea antidysntrica* extracts/fractions and positive reference standards are shown in figure 5. As shown in figure 5, the reducing power of all the extracts/fractions gradually increased with increasing concentration of the extracts. This indicated that all extracts possessed the ability (either strong or weak) to reduce $Fe^{3+}$/ ferric cyanide complex to the ferrous form when being evaluated by reducing power assay. However, the reducing power of the positive reference standards (ascorbic acid) was relatively more pronounced than the tested extracts and fractions.

**Table 8: The reducing power of different extracts and fractions expressed as mg ascorbic acid equivalent per gram sample.**

| Plant extracts/fractions | Antioxidant power ( mg AAE/g dry of extract) |
|---|---|
| 80% methanol extract | 52.05 ± 0.02 |
| Absolute methanol extract | 25.51 ± 0.08 |
| Chloroform extract | 14.88 ± 0.10 |
| Petroleum ether extract | 2.37 ± 0.03 |
| **Fractionations of 80%methanol crude extract** | |
| Aqueous fraction | 72.40 ± 0.22 |
| Chloroform-mehanol fraction | 68.10 ± 0.09 |
| Chloroform fraction | 37.42 ± 0.18 |

The reductive capability of the extracts and its fractions decreases in the following order: aqueous fraction > chloroform-methanol fraction > 80% methanol > chloroform fraction > absolute methanol > chloroform > petroleum ether extract.

The positive control, ascorbic acid, demonstrated much higher reducing power compared to the crude extract and its fractions even at low concentration (Figure 5). The aqueous fraction of 80% methanol extract showed the highest reducing activity among the extracts and fractions of *Brucea antidysntrica*. The results presented revealed that the aqueous fraction has the highest electron donating capacity possibly contained higher amounts of reductones. The aqueous fraction and chloroform-methanol fraction possessed lower reducing activity compared to the positive reference standards while the petroleum ether extract showed the lowest reducing power (Figure 5). The aqueous fraction and chloroform-methanol soluble fraction of *brucea antidysntrica* may be found to contain mainly polar alkaloides, saponins, and highly polar metabolites like polyphenolics, glycosides, and quaternary alkaloides.

It is highly probable that the lone pairs of electron on nitrogen (alkaloids), and the carbonyl oxygen of the polyphenol can be easily donated to the ferric ions in the reducing power assay.

## Radical scavenging activity (DPPH˙)

In the DPPH scavenging assay, crude extracts and fractions of this plant were investigated through the free radical scavenging activity *via* their reaction with the stable DPPH radicals. The reduction of the DPPH was followed *via* the decrease in absorbance at 517 nm. The various crude extracts and fractions of *Brucea antidysntrica* significantly reduced the DPPH. The free radical scavenging activities (%) of extracts and fractions as DPPH in the following decreasing order as aqueous fraction (87.57 ± 1.15) > chloroform-methanol fraction (84.84 ± 0.62) > 80% methanol extract (81.59 ± 0.21) > absolute methanol extract (79.43 ± 0.14) > chloroform fraction (77.75 ± 0.11) > chloroform extract (74.86 ± 0.18) > Petroleum ether extract (68.85± 0.13). It was observed that the DPPH scavenging activity increased with increasing concentration of the fractions in the assay. For the various concentrations, soluble aqueous fraction exhibited the highest percent inhibition of the DPPH as compared to the other extracts and fractions. This fraction showed 87.57 ± 1.15 % inhibition of DPPH at a concentration of 100 μg $mL^{-1}$. Antiradical activity increases with increasing polarity of solvent, Hydro-alcoholic, 80% methanol extract (81.59 ± 0.21) showed the highest scavenging activity of the crude extracts. The positive control, ascorbic acid showed maximum scavenging effect at very low concentration (Figure 6).

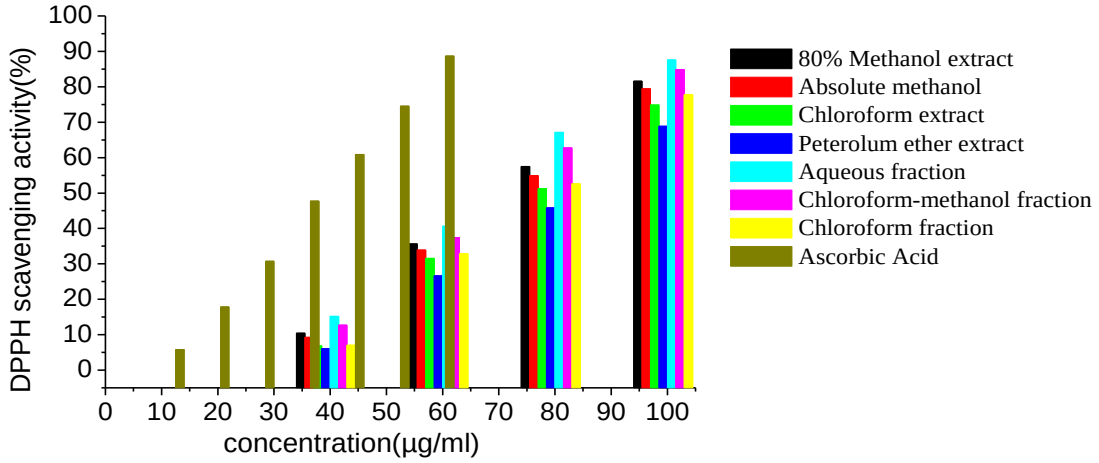


**Figure 6**: DPPH scavenging activity (%) of *Brucea antidysntrica* extracts/fractions and positive reference standard (ascorbic acid).

**Table 9**: **The radical scavenging activity ($IC_{50}$ Values (mg/ml) of the leaf of *Brucea antidysntrica* extracts/fractions on DPPH radicals.**

| Plant extracts | $IC_{50}$ Values (mg/ml) |
|---|---|
| 80% methanol extract | 0.073 ± 0.03 |
| Absolute methanol extract | 0.074 ± 0.08 |
| Chloroform extract | 0.082 ± 0.01 |
| Petroleum ether extract | 0.085 ± 0.05 |
| **Fractionations of 80%methanol crude extract** | |
| Chloroform fraction | 0.078 ± 0.01 |
| Chloroform-methanol fraction | 0.070 ± 0.02 |
| Aqueous fraction | 0.067 ± 0.04 |
| Ascorbic acid (Vitamin-C) ($IC_{50}$ = 0.034 ± 0.11 mg/mL). | |

*Values expressed are mean ± standard deviation of three measurements. $IC_{50}$ value is defined as efficient concentration of DPPH radical being scavenged by 50%.*

The $IC_{50}$ value is defined as the concentration of a substrate that causes 50 % loss of the DPPH activity and was calculated by linear regression of plots of the percentage antiradical activity against the concentration of the tested compounds.

The radical scavenging activity ($IC_{50}$ values) of extracts, fraction and positive reference standards on DPPH radicals are shown in Table 9. The lower the $IC_{50}$ value, the higher is the scavenging potential. The soluble aqueous fraction exhibited the lowest $IC_{50}$ value, *i.e.*, 0.067 ± 0.04 mg/mL as compared to the other studied extracts and fractions.

The highest $IC_{50}$ value was found in petroleum ether which is a non polar solvent and exhibited the weakest scavenging effect. The results are expressed relative to ascorbic acid (Vitamin-C), reference standard having $IC_{50}$ of 0.037 ± 0.11 mg/mL. Generally, in comparison to both positive reference standards, ascorbic acid ($IC_{50}$ = 0.037± 0.11 mg/mL), all the extracts and fractions displayed moderate scavenging activity, except petroleum ether extracts which exhibited the weakest scavenging effect (0.085 ± 0.05 mg/mL; **Table 9**). The lower $IC_{50}$ value for the aqueous fractions as compared to the crude extract coupled with their higher free radical scavenging activity showed that the fractions have more bioactive compound as compared to its crude 80% methanol extract. The result here revealed that extracts of *Brucea antidysntrica* can be considered as free radical inhibitors or scavengers. The capacity of plant extracts to act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators or free radical scavengers indicates their potential therapeutic properties for treating diseases related to free radical reactions.

## Correlations among total phenolics, total flavonoids and antioxidant capacity

The combination of four methods applied in this study gave valuable information in the evaluation of the antioxidant activity of *Brucea antidysntrica* extracts. Regression and correlation analysis were performed to determine relationship between these parameters (**Figure** 7 and 8).

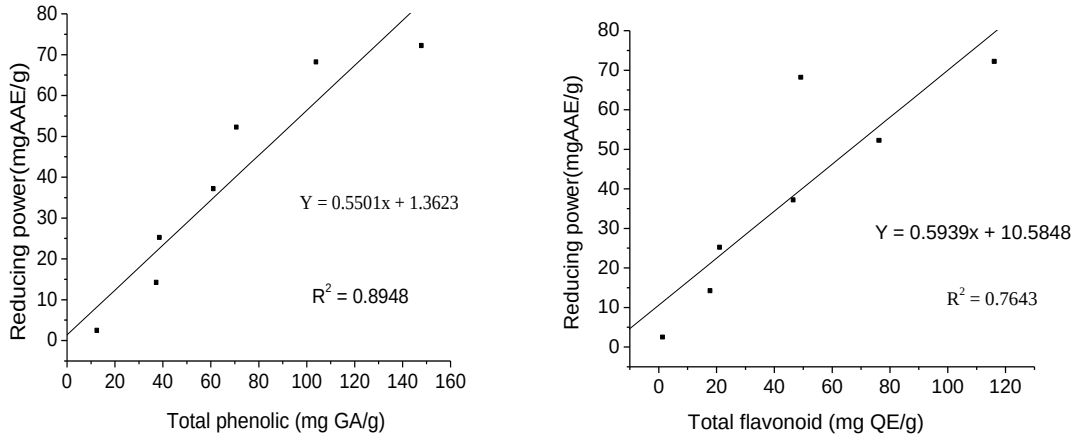


**Figure 7:** Correlation between reducing power (mg AAE/g) and total phenolic (mg GAE/g) contents and with total flavonoid (mg QE/g) contents.

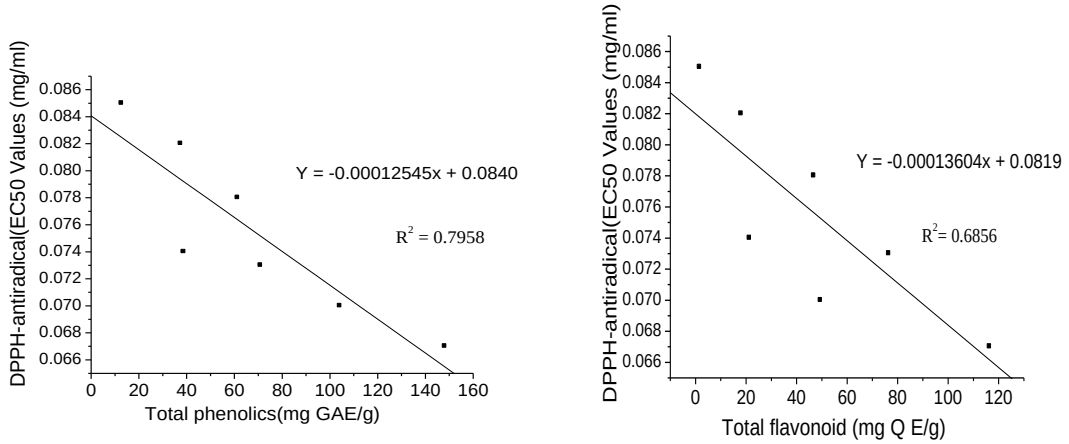


**Figure 8: Correlation between free DPPH radical scavenging activity ($IC_{50}$) and total phenolic contents and with total flavonoid contents.**

The reducing power of different solvent extracts and fractions were expressed as mg ascorbic acid equivalent per gram sample and its results were shown in Table 8.

Among fractions evaluated, aqueous fraction has the highest reducing power, which was correlated with the total phenolic content and total flavonoid content. The correlation between reducing power activity of fractions and its phenolic content revealed an equation: y = 0.5501x + 1.3623 and $R^2$ = 0.8948; while with the flavonoid content showed: y = 0.5939x + 10.5848; $R^2$ = 0.7643. Taking into account of $R^2$ values, these results suggested that phenolic compounds more likely contribute to its reducing activity than do flavonoids and also there is strong correlation between the FRAP with TPC and TFC indicated that phenolic and flavonoid compounds present in the plant extracts capable to reduce $Fe^{3+}$ to $Fe^{2+}$ as a result that the bioactive substances found in *Brucea antidysenterica* leaf are power full reducing agents to free radicals.

The relationship between free radical scavenging activities(Y) with total phenolic content (X) revealed coefficient of determination ($R^2$) of 0.7958, whereas with total flavonoid content (X) has $R^2$ of 0.6856.

These results suggested that phenolic compounds and flavonoid compounds contributed 79.58 % and 68.56 % to free DPPH radical scavenging of the crude extracts and fractions from *Brucea antidysntrica* leaf. The correlation between DPPH with TPC and TFC showed that there is positive relation between DPPH with TPC and TFC as a result the leaf of *Brucea antidysenterica* can able to scavenge free radicals. However, DDPH value is more correlated with TPC than TFC. Also, it can be stated that scavenging effect of extracts/ fractions is not limited to phenolics and flavonoid compounds. Thus, the activity may also come from the presence of other antioxidant secondary metabolites in the extracts such as volatile oils, carotenoids, and vitamins.[24]

In the study the ferric reducing power is better to evaluate the *Brucea antidysenterica* leaf extract than DPPH radical scavenging assay. The probable reason is due to the slow reaction of DPPH radical with both electron and hydrogen donor metabolites. The slow reaction of DPPH is due to steric effect to the radical site. Generally, this study confirmed that *Brucea antidysntrica* can be a significant source of natural antioxidant that may have potent beneficial health effects.

## CONCLUSION

The total phenolic, flavonoid and antioxidant activity of *Brucea antidysntrica*s leaf were evaluated using the Folin-Ciocalteu reagent, aluminum chloride colorimetric assay, FRAP and DPPH assay respectively. There is strong correlation between these four parameteric values. The results of the current study showed that all the extract and fractions of *Brucea antidysntrica*s exhibited different extent of antioxidant activity. The 80% ME and its fractions, which contain the highest amount of phenolic content, exhibited the greatest antioxidant activity *in vitro* models. Thus, this study suggests that the aqueous alcoholic fraction of *Brucea antidysntrica*s can a potential source of natural antioxidant. The finding of this study supports that *Brucea antidysntrica*s plant is a promising source of antioxidants for utilization in pharmaceutical fields as reducing agent.

## RECOMMENDATION

The combination of four methods applied in this study gave valuable information in the evaluation of the antioxidant activity of *Brucea antidysntrica*s extracts and could be recommended for other similar investigations. Further studies are warranted for the isolation and characterization of antioxidant compounds, and also *in vivo* studies are needed for understanding their mechanism of action as antioxidants. The people can use as strong antioxidant activity to provide a homecare herbal remedy against bacterial infections, they should preserve this plant as indigenous botanical and medicinal knowledge through active use, and preserving their cultural and spiritual values passing to future generation.

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