

Full Length Research Paper

Phytochemical screening and peroxide value determination of methanolic extract of four traditional medicinal plants from Debre Tabor Town, Ethiopia

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Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing of different disease. Terpenoids, flavonoids, alkaloids, tannins are some of bioactive compounds present in different parts of medicinal plants. Qualitative identification of bioactive compounds and antioxidant activity determination of crude extracts of the leaves of four different medicinal plants (*Cordia africana* Lam, *Croton macrostachyus* Hochst, *Vernonia amygdalina* Del. and *Justicia schimperiana* T.Anders.) based on peroxide value (POV) method were carried out in this study. For the phytochemical screening test, the results confirmed the presence of polyphenols, tannins, alkaloids, steroids and anthraquinones in all plants leave extracts, however flavonoids, glycosides and phlobatannins were absent. In case of peroxide value (POV) determination, the lowest value were recorded in crude extracts of *Cordia africana* Lam leaves (24 meq/kg) and the highest value were observed in *Justicia Schimperiana* leaves extracts (101 meq/kg) in the 1st treatment. In the 4th treatment, *Schimperiana* T. Anders had lowest antioxidant activities [highest POV (290 meq/kg)] and *C. Africana* had the highest antioxidant activities [lowest POV (88meq/kg)]. The study also showed that temperature variations had its own influence on peroxide value. As the temperature of the system increased, the peroxide content of sample treated with leave extracts of plants also increased, however the degree of increment vary within different temperature range (30 to 50°C, 50 to 75°C and 75 to 100°C). In all temperatures, the peroxide production of niger seed oil containing plants leave extracts were less than oil free from extracts. The data generally indicates that the POV of plants leave extract showed antioxidant activity due to the presence of some Phytochemical (polyphenols, tannins, glycosides) present in plants leave extracts.

Key words: Medicinal plants, phytochemical, antioxidant, peroxide value (POV).

INTRODUCTION

Plant species still serve as a rich source of many novel biologically active compounds. The interest in phytomedicine and many medicinal plant species are being screened for biological activities (Mohammad, 2015). The World Health Organization (WHO) reported that 80% of the emerging world's population relies on traditional medicine for therapy. During the past decades,

the developed world has also witnessed an ascending trend in the utilization of complementary and alternative medicine (CAM), particularly herbal remedies (Manish et al., 2015). Traditional medicinal plants are widely used in different part of the world for curing diseases. For instance, in China, about 30 to 50% of the total medicinal consumptions was obtained from traditional herbal

preparations. In Africa, Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children in Ethiopia up to 80% of the population uses traditional medicine due to the cultural acceptability of healers and local pharmacopeias, the relatively low cost of traditional medicine and difficult access to modern health facilities (Ahmed et al., 2016).

Traditional medicinal plants are rich in bioactive compounds. Medicinal and aromatic plants contain biologically active chemical substances such as saponnines, tannins, essential oils, flavonoids, alkaloids and other chemical compounds, which have curative properties. These chemical organic compounds of different compositions are found as secondary metabolites in one or more of these plants (Ammar et al., 2017). The presence of bioactive compounds in their leaves, root, stem, bark make traditional plants to have antioxidant properties and free radical scavenging activities (Mamta et al., 2013). The antioxidant properties of the plant were measured using different assays; among the methods used was the determination of peroxide value of the plant extract (Zaid et al., 2013). The peroxide value (POV) is the number that expresses in mill equivalents of active oxygen the quantity of peroxide contained in 1000 g of the substance; it provides information regarding the antioxidant activity of substances by measuring the oxidative stability of oil (Wahid et al., 2015; Andina et al., 2017).

Cordia Africana Lam, *Croton macrostachyus* Hochst, *Vernonia amygdalina* Del and *Justicia schimperiana* T. Anders are some of the traditional plants found in Ethiopia and outside the country. These different vegetation types are found in the various agro ecological zones of Ethiopia (Mirutse et al., 2006). The wood lands, Montane vegetation including grasslands and forests and the evergreen scrubs and rocky areas contain those medicinal plants with higher concentrations in the wood lands (Asefa et al., 2014). The research made so far on these plants in Ethiopian has been mostly of producing inventories and checklists; scientifically the antioxidant activities have not been investigated. The objectives of this paper were to investigate the antioxidant activities of the plants leave extract based on the peroxide value (POV) methods and qualitative phytochemical determination of some bioactive compounds found in plant leave extract in the study area.

MATERIALS AND METHODS

Collection of plant materials

The medicinal plants leaves of *C. africana* Lam, *C. macrostachyus*

Hochst, *V. amygdalina* Del and *J. schimperiana* T. Anders were identified by botanist Mr. Haileab Zegeye around Debre Tabor town and the leave of these plants were collected, washed properly with tap water and dried at room temperature (23°C) without sun light. The dried plants leaves were powdered by coffee grinder (instruments used to grind the dried leaves) and these powdered leaves were stored in a clean polyethylene bags until extraction was carried out in Debre Tabor University chemistry laboratory.

Extraction procedure

Two hundred milliliter methanol were added to 20 g powdered leaves of *C. Africana* Lam, *C. macrostachyus* Hochst, *V. amygdalina* Del and *J. schimperiana* T. Anders in a separate conical flask and shaken with electrical shaker for 48 h. Each solution was filtered by using Whatman number 1 filter paper in a separate conical flask. After separation, each extracts labeled as MCA, MCM, MAA and MJS to represent methanol extract of *C. africana* Lam, *C. macrostachyus* Hochst, *V. amygdalina* Del and *J. schimperiana* T. Anders respectively. Each extracts were kept in cool and shaded place until further experiments were takes place.

Phytochemical screening procedures

Test for phlobatannins

Extract of each plant leave powder were boiled separately with 1% aqueous hydrochloric acid and then deposition of a red precipitate in flask confirms the presence of phlobatannins (Sriyeta et al., 2017).

Test for carotenoids

One gram of each extract sample was mix with 10 ml of chloroform in a separate test tube with vigorously shaking. After the mixture, the extract was filtered with filter paper and 85% sulphuric acid was added. Blue color at the interface was present which indicates the presence of carotenoids (Sriyeta et al., 2017).

Test for quinones

To one millilitre of the extract, 1 ml of concentrated sulphuric acid was added. Formation of red color shows the presence of quinines (Sriyeta et al., 2017).

Test for xanthoproteins

One millilitre each of the various extracts was treated separately with few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitate indicates the presence of xanthoproteins (Sriyeta et al., 2017).

Test for polyphenols and tannins

Crude extract was mixed with 2 ml of 2% solution of FeCl_3 . A blue-

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green or blue-black coloration indicated the presence of polyphenols and tannins (Singh et al., 2015; Devi, 2015).

Tests for polyphenols

One millilitre of each crude extract of the sample was mixed with 2 ml of 2% solutions of ferric chloride. The black color indicates the presences polyphenols (Singh et al., 2015).

Tests for tannins

Two millilitres of each crude extracts of the sample was taken in a separate test tube; each sample of solution was stirred with 2 ml of distilled water. Three drops of ferric chloride solutions were added to each sample of solution. The formation of green precipitate was an indication for the presence of tannins (Singh et al., 2015; Devi, 2015).

Tests for glycosides

Two millilitres of each organic extract was dissolved in 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added carefully and shaken gently for each sample of solutions. The reddish brown color indicates the presence of Steroidal ring (that is a glycone portion of glycoside) (Sriyeta et al., 2017).

Test for flavonoids

Three drops of 1% of NH_3 or NaOH solution was added to each of methanol extracts of the plant leaves sample in a test tube. A yellow coloration was observed to confirm the presence of flavonoid compounds (Yusuf et al., 2013).

Tests for saponnins

Two millilitres of each methanol crude extracts was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam indicates the presence of saponnins (Sriyeta et al., 2017).

Tests for steroids

Two millilitres of each methanol crude extracts was mixed with 2 ml of chloroform and 2 ml of sulphuric acid to each sample of solution side wisely. The development of greenish coloration indicates the presence of steroids.

Tests for terpenoides

Two millilitres of each methanol organic extract was dissolved in 2 ml of chloroform in a separate test tube. Each sample solution was dried by hot plate. 2 ml of concentrated sulphuric acid was added to each sample of solution. Each sample of solution was heated for 2 min. Grayish color formation indicates the presence of terpenoides.

Tests for anthaquinone

Two millilitres of each methanol organic extract was mixed with 2 ml of benzene. 3 ml of 1% NH_3 solvent was added to the sample of plant extract. The violet color shows the presence of anthraquinone (Sriyeta et al., 2017).

Tests for alkaloids

Two ml of each of methanol organic extract was stirred with 2 ml of 1% of HCl on four drops of Wagner's reagent. The turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids (Singh et al., 2015).

POV procedure

For peroxide value determination, Niger seed oil (NSO) was used because it is a valued source of edible oil in Ethiopia and contains linolic acid (important chemical for antioxidant research) in it (Gashaw and Getachew, 2014; Getachew, 2014). Six different samples *C. Africana* Lam leave extract and NSO, *C. macrostachyus* Hochst leave extract and NSO, *Vernonia amygdalina* Del leave extract and NSO, *J. schimperiana* T. Anders leave extract and NSO, ascorbic acid (AA) and NSO and Niger seed oil only (control) were prepared for determination of peroxide value. Six of them were placed at room temperature (23°C) for three week using four different treatments (treatment 1, treatment 2, treatment 3 and treatment 4) with five days interval to test their POV. For thermal effect test, some portion were taken from six samples that were placed at room temperature and kept at 30, 50, 75 and 100°C. From each sample, 5 g were taken and added to different 250 ml conical flask. 30 ml of a mixture of glacial acetic acid and chloroform (3:2) were added to each sample. The mixtures were shaken to dissolve, and 0.5 ml of saturated potassium iodide solution were also added to each flask which were placed at room temperature (23°C) and at 30, 50, 75, 100°C and then the mixture were shaken for 1 min. Finally, 30 ml of water were added to each sample and titrated with 0.01 N sodium thiosulfate solutions. After the yellow color disappears, 5 ml of starch solutions were added to each sample to indicate the end of titration. The titrant was added slowly with continuous shaking, until the blue color was discharged. A blank determination was performed under the same condition.

Statistical analysis

All measurements were carried out in triplicate ($n=3$), and values expressed are the mean of three measurements. Results were analyzed statically by using Excel 2007 and the graphs were displayed using origin 8 software, and difference between mean were determined by the least significant difference test, and significance was defined as a confidence limit of $P < 0.05$. The peroxide value (meq/kg oil) plants leave extracts against the niger seed oil was calculated with equation (1) (Adejumo et al., 2013).

$$\text{POV (meq/kg)} = \frac{(S-B)(C)(1000)}{m} \quad (1)$$

Where, S = volume of titrant ($\text{Na}_2\text{S}_2\text{O}_3$) needed for sample, B = volume of titrant ($\text{Na}_2\text{S}_2\text{O}_3$) needed for blank, C =Concentration of titrant ($\text{Na}_2\text{S}_2\text{O}_3$), and m = mass of plant leave extract.

RESULTS AND DISCUSSION

Preliminary phytochemical test

Preliminary qualitative phytochemical analysis of methanolic extract of the plants leaves showed the presence of different groups of secondary metabolites. In all tested plants leaves extracts, polyphenol, tannins, steroids, anthraquinones were present; however

Table 1. Results of phytochemical test of four different plants leave extracts (methanolic).

Phytochemicals	Types of medicinal plants			
	<i>Cordia africana</i> Lam	<i>Croton macrostachyus</i> Hochst	<i>Vernonia amygdalina</i> Del.	<i>Justicia schimperiana</i> T. Anders
Polyphenol	+	++	++	++
Tannins	++	++	++	++
Glycosids	-	++	-	-
Flavonoids	-	-	-	-
Terpenoids	-	-	-	++
Alkaloids	+	+	-	-
Saponnins	++	-	-	-
Steroids	++	++	+	+
Anthraquinons	++	+	+	++
Phlobatannins	-	-	-	-
Carotenoids	-	+	-	+
Quinones	++	-	-	-
Xanthoproteins	-	++	+	++

Key ++ = strong, + = medium, - = absent.

flavonoids and phlobatannins were absent. Quinones were detected only in *C. Africana* Lam leave extracts and glycosides were present only in *C. macrostachyus* Hochst leave extracts. Compared to all other leave extracts, the leaves of *V. amygdalina* Del. contained few secondary metabolites as showed in Table 1 above.

Peroxide value (POV) determination

Peroxide value is widely used to measure the primary lipid oxidation formed in fats and oils during oxidation (Wsowicz et al., 2014). The peroxide value of blank Niger seed oil sample was 105 meq/kg in the first treatment. It was increased to 295 meq/kg at the end of the treatment. These changes significantly indicate the noticeable phenomenon of lipid oxidation. Peroxide values of Niger seed oil containing MJS were found to be 101 meq/kg in the first treatment; it was 292 meq/kg at the completion of treatment. Investigations in the case of methanol extract of *C. Africana* Lam containing Niger seed oil samples expressed the peroxide value which increased from 24 meq/kg (first treatment) to 88 meq/kg (last treatment). POV of Niger seed oil containing *V. amygdalina* Del. changed from 72 to 152 meq/kg and the peroxide value of Niger seed oil that contain *C. macrostachyus* Hochst varied from 89 to 212 meq/kg.

There was increase in peroxide values (POV) of Niger seed oil containing MCA, MCM, MVA and MJS during all treatment. The increment between each treatment was highest in MJS and lowest in MCA (Figure 1). This shows that there was a variation in increasing of primary products (peroxides) and secondary products (aldehydes and ketones) in each plant leave extracts (Monika et al., 2014).

According to the graph and analyzed data, the POV of control was significantly higher, than the POV of almost all other treatments. Treatment containing MJS was significantly higher in POV than the treatment containing MCA, MCM, MAA and AA. Treatment containing AA maintained a significantly lower POV than almost all other in the first and second treatments. Treatment containing *C. Africana* Lam maintained the lowest POV for third and fourth treatment (Figure 1).

The above observations show that oxidation of Niger seed oil was highly hindered by the treatment containing AA and MCA, and oxidation of niger seed oil least hindered by the treatment containing MJS. According to these POV value, AA and MCA had the highest antioxidant activity and the least antioxidant activities were recorded in MJS (highest peroxide value).

The POV variations were statistically significant between AA, MCA, MJS, MVA and MCM in each treatment; however there were no significant variations between control and MJS and AA and MCA. The POV variation between niger seed oil containing AA and MCA and niger seed oil containing MJS extracts and control were very small. These showed that the antioxidant activities of AA and *C. Africana* Lam had almost identical (the highest value) and MJS had almost the same antioxidant power with control (lowest value).

The study also showed that temperature variations had its own influence on peroxide value. As the temperature of the system increased, the peroxide content of sample treated with contain MJS, MCA, MVA, MCM, AA and control also increased, however the degree of increment varied within different temperature range (30 to 50°C, 50 to 75°C and 75 to 100°C). The peroxide value difference between 50 to 75°C was the highest and the lowest peroxide value difference was recorded between 75 to

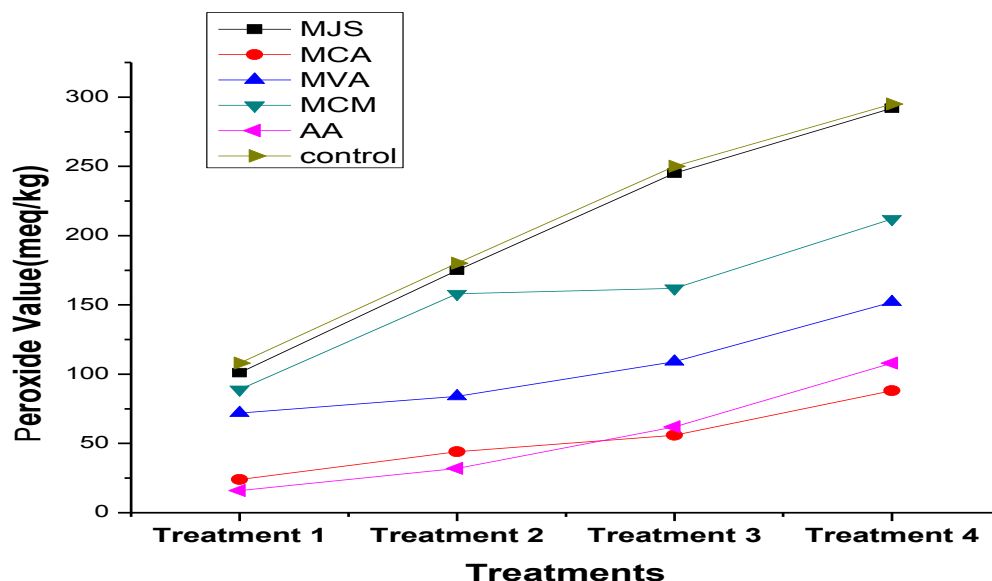


Figure 1. Changes in peroxide value (meq/kg) during storage at room temperature (23°C) for three week.

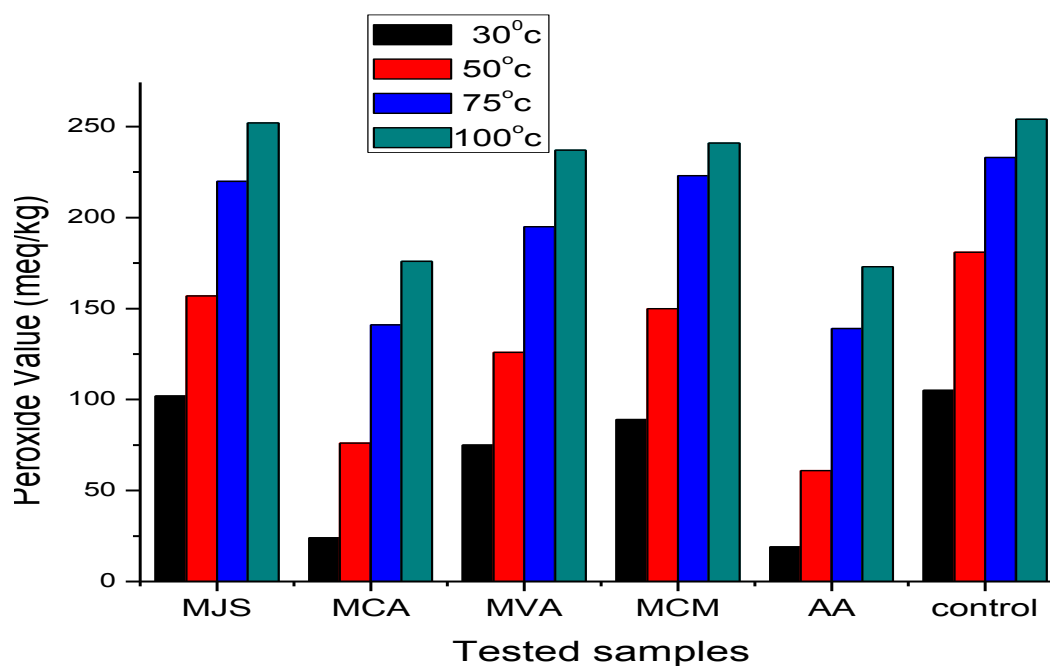


Figure 2. POV Comparison between different Levels of heat on methanolic extracts of leave of four traditional medicinal plans.

100°C temperature ranges (Figure 2). This showed that the increment of temperature above 75°C had its own negative impact on antioxidant activities of the plants leave extracts. Treatment containing *C. africana* Lam and ascorbic acid had the lowest POV than other treatments

at all tested temperature. The lowest peroxide values were recorded at 30°C and the highest peroxide value were obtained at 100°C in all treatment. At all temperatures tested, the peroxide production of Niger seed oil containing MJS, MCA, MVA, MCM and AA was

less than oil free from extracts. The present study also shows that increasing temperature accelerates the oxidation rate of Niger seed oil and decreases the antioxidant properties of the extracts.

Conclusion

Phytochemicals present in leave extracts of *C. africana*, *V. amygdalina*, *J. schimperiana*, and *C. maroactachyus* indicates their potential as a source of antioxidant. The distribution and content of phytochemicals differ in medicinal plants. According to the POV value, plants leave extract showed antioxidant activity due to the presence of some phytochemicals, however the antioxidant activities varies from plant to plant and the power of antioxidant influenced by thermal variation. As thermal temperature increase, the antioxidant power of the plants leave extract decrease and this might be because the heat applied would decompose some volatile and less stable metabolites that are used to reduce oxidation. Furthermore, isolation purification and characterization of the phytochemicals present in plants will make interesting studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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