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Full Length Research Paper

Antioxidant activities of the selected plants from the family Euphorbiaceae, Lauraceae, Malvaceae and Balsaminaceae

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Extraction of nine plants selected from the family Euphorbiaceae, Lauraceae, Malvaceae and Balsaminaceae was done in petroleum ether, chloroform, ethyl acetate and methanol/n-butanol in order of increasing polarity using soxhlet apparatus. Total phenolic contents were determined with Folin-Ciocalteu reagent which ranged from 30.5 to 547.0 mg GAE/g of extract. Maximum phenolic contents were found in n-butanol extract of *Ricinus communis*. Antioxidant activities of these extracts were evaluated through DPPH$^{\cdot}$ radical scavenging, phosphomolybdate and ferric thiocyanate (FTC) methods. Methanolic extract of *Cinnamomum zeylanicum* and *Cinnamomum tamala* showed highest antiradical (96.8%) and phosphomolybdate (1.131) activity, respectively, while ethyl acetate extract of *R. communis* exhibited maximum lipid per-oxidation (FTC) activity (79.3%). IC_{50} value of chloroform extract of *C. tamala* (2.2 µg/ml) was less than gallic acid (4.4 µg/ml), while ethyl acetate and methanol extracts of *Abutilon bidentatum*, *Impatiens bicolor* and *Impatiens edgeworthii* exhibited the IC_{50} values in the range of 10.0 - 20.0 µg/ml.

Key words: Euphorbiaceae, Lauraceae, Malvaceae, Balsaminaceae, antioxidant assays, medicinal plants, total phenols.

INTRODUCTION

Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical-induced tissue injury (Pourmorad et al., 2006). The major constituents of biological membranes are lipids and proteins. Reactive oxygen species can easily initiate damage of the cell membrane constituent that is, phospholipids, lipoproteins by propagating a reaction cycle (Raja et al., 2006; Prakash et al., 2009). It has been mentioned by many authors that antioxidant activity of plants is due to their phenolic compounds (Duh et al., 1999; Dragland et al., 2003; Wang, 2003; Wu et al., 2004).

Jatropha gossypifolia (Euphorbiaceae) has anticancer, hepatoprotective and pesticidal activity. The leaf decoction of this plant is used for bathing wounds (Sosa et al., 2002; Kayaalp, 1998; Hartwell, 1969; Chatterjee et al., 1980; Panda et al., 2009). *Chrozophora tinctoria* (Euphorbiaceae) is used in dyeing (Paolo, 2006), *Euphorbia royleana* (Euphorbiaceae) has anti-inflammatory, acetylcholinesterase and piscicidal activity (Sudhanshu and Ajay, 2004), while *Ricinus communis* (Euphorbiaceae) commomnly known as castor oil tree, is widely used as a human laxative-cathartic agent, particularly in cases of certain radiological examinations which require prompt and thorough evacuation of the small intestine (Fingl, 1980), *Abutilon bidentatum* (Malvaceae) has antibacterial, central nervous system (CNS) depressant and antidiabetic activities (Aderotimi and Samuel, 2006; Lakshmayya et al., 2003). *Cinnamomum tamala* and *Cinnamomum zeylanicum* (Lauraceae) have analgesic, antiseptic, antispasmodic, aphrodisiac, astringent, carminative, insecticidal and parasiticidal activities (Burkill, 1966). *Impatein bicolor* and *Impatein edgeworthii* (Balsaminaceae) show a long lasting skin moisturizing effect and prevent dryness, rough skin chap, used to prepare lotions, creams, hair tonics, cosmetics, bath preparations and detergents (Hassan and Tahir, 2005; Charles and

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Hagen, 1996).

In longer term, plant species identified as having high levels of antioxidant activity *in vitro* may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals-induced tissue damage. A number of synthetic antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) have been added to foodstuffs but, are reported to cause liver disorders (Valentao et al., 2002). Therefore, attention has been directed towards the purification of natural antioxidants from botanical sources, especially edible plants. The study was conducted to evaluate the antioxidant activity of the nine medicinal plants from four different families using different antioxidant models (ferric thiocyanate (FTC), 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and phosphomolybdate).

MATERIALS AND METHODS

Materials

Folin-Ciocalteu (FC) reagent, DPPH, BHT, α -tocopherol, gallic acid, linoleic acid and potassium thiocyanate were purchased from Fluka and Sigma-Aldrich (USA). All other chemicals and solvents were analytical grade.

Preparation of plant extracts

Whole plants of family Euphorbiaceae and Malavaceae were collected from Lahore region; plant material of Balsaminaceae was collected from Ayubia park, Muree (Pakistan) and identified at the Department of Botany (GC University, Lahore), while the leaves of *C. tamala* and bark of *C. zeylanicum* (Lauraceae) were purchased from local market. All plant materials were dried, powdered and extracted in different solvents (petroleum ether, chloroform, ethyl acetate and methanol/ n-butanol) using soxhlet apparatus. Crude extracts were filtered and concentrated at reduced temperature using rotary evaporator.

Determination of total phenols

Total phenolic contents of all the extracts were determined by Folin-Ciocalteu reagent (Makkar et al., 1993). 0.1 ml of extract was combined with 2.8 ml of 10% Na_2CO_3 and 0.1 ml of 2 N Folin-Ciocalteu reagent. After 40 min, absorbance was measured at 725 nm using UV-visible spectrophotometer (CECIL-7200). The results were determined as mg equivalent of gallic acid per gm of extract by computing with standard calibration curve (R^2 = 0.9909 value) constructed for different concentrations of gallic acid.

FTC assay

The antioxidant activity of different extracts on inhibition of linoleic acid per oxidation was assayed by ferric FTC (Osawa and Namiki, 1981). 0.1 ml of the ethanolic solution of the extract (5 mg/ml) was mixed with 10 ml of absolute ethanol, 10 ml of 0.2 M phosphate buffer (pH 6.0) and 2 ml of 2% (v/v) linoleic acid. All the samples were incubated at 40°C. At regular intervals, (48 h) 5 ml ethanol, 0.1 ml 0.02 M ferrous chloride in 3.5% HCl and 0.1 ml of aq. 20% ammonium thiocyanate was added in the above solution and absorbance was recorded at 500 nm. Gallic acid, BHT and α - tocopherol were used as standard reference in 2 mg/ml concentration.

DPPH radical scavenging assay

The antioxidant activity of different extracts was measured in terms of radical scavenging ability by DPPH method (Erasto et al., 2004). Methanolic solution (1 ml) of different extracts at 100 µg/ml concentration was added to 1 ml methanolic solution of DPPH (2 mg/ml). The absorbance was measured at 517 nm after 30 min. The results were evaluated as percentage scavenging of radical (% scavenging of $DPPH^{\bullet}$ = Abs. of blank - Abs. of sample/ Abs. of blank x 100). IC_{50} value (concentration of sample where absorbance of DPPH decreases 50% with respect to absorbance of blank) of extracts were determined. The results were compared with standards (gallic acid and BHT).

Phosphomolybdate assay

Total antioxidant activity of extracts was evaluated by the formation of phosphomolybdenum complex (Prieto et al., 1999). 0.1 ml methanolic solution of extracts (100 µg/ml) was added to 1.9 ml of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The blank solution contained only 2 ml of reagent solution. The absorbance was measured at 695 nm after 60 min.

RESULTS AND DISCUSSION

Total phenolic contents

Phenolic compounds are commonly present in both edible and non-edible plants and exhibit multiple biological effects including antioxidant activity (Kahkonen et al., 1999). The phenolic contents of the selected plant extracts were determined by FC reagent and expressed as gallic acid equivalents in mg/g of crude extract. Total phenolic contents of different solvent extracts are given in Table 1. The yields ranged from 30.5 to 547.0 mg GAE/g of crude extracts. All the ethyl acetate and methanol/ n-butanol extracts were found rich in phenolics except the extract ERE of *E. royleana* and ChTB of *C. tinctoria* which showed 88.5 and 64.5 mg GAE/g of crude extract. Among the other extracts, n-butanol extract of *R. Communis* (RCB) demonstrated the highest phenolic content (547.0 GAE/g of crude extract).

Antioxidant activities

DPPH radical scavenging assay

Antioxidants react with $DPPH^{\bullet}$, which is a stable free radical and convert it to 1, 1′-diphenyl-2-picryl hydrazine (Fargere et al., 1995). The degree of decolourization of the purple coloured solution of $DPPH^{\bullet}$ indicated the scavenging potential of the antioxidant compound. It was found that the radical scavenging activity of CZM, CTP,

Table 1. % Scavenging of DPPH, % lipid per oxidation, total phenols and total antioxidant activity of the extracts of selected medicinal plants.

Extracts/Standards (100 µg/ml)	Total phenols (mg GAE/g of crude extract)	% Scavenging of DPPH	Total antioxidant activity	% Inhibition of lipid per oxidation	IC_{50} (µg/ml)
ABP	104.6 ± 1.4	25.5 ± 0.2	0.323 ± 0.008	23.4 ± 1.1	40.0
ABC	257.3 ± 1.9	62.3 ± 0.5	0.401 ± 0.016	39.8 ± 2.5	26.0
ABE	532.6 ± 2.6	88.5 ± 0.4	0.483 ± 0.012	78.4 ± 2.0	10.0
ABM	495.2 ± 3.5	76.9 ± 0.7	0.217 ± 0.015	71.9 ± 1.8	16.0
IBP	104.6 ± 0.9	33.9 ± 0.9	0.231 ± 0.017	34.2 ± 1.3	-
IBC	257.3 ± 1.2	45.0 ± 1.1	0.304 ± 0.019	52.4 ± 2.1	-
IBE	429.4 ± 3.7	92.2 ± 1.4	0.495 ± 0.023	68.3 ± 2.6	11.0
IBM	492.2 ± 4.4	88.2 ± 1.0	0.505 ± 0.027	68.3 ± 2.9	19.0
IEP	125.2 ± 0.5	32.5 ± 0.5	0.263 ± 0.019	29.7 ± 1.0	-
IEC	184.6 ± 1.1	33.7 ± 0.4	0.384 ± 0.018	49.9 ± 1.7	-
IEE	429.4 ± 1.8	71.0 ± 0.9	0.473 ± 0.023	72.3 ± 1.4	17.0
IEM	359.2 ± 2.4	66.9 ± 1.2	0.342 ± 0.011	71.8 ± 2.2	20.0
CTP	272.4 ± 3.0	94.3 ± 1.6	0.885 ± 0.039	44.4 ± 0.9	55.0
CTC	273.7 ± 1.6	88.5 ± 1.3	0.923 ± 0.064	49.2 ± 1.4	2.2
CTE	271.0 ± 2.2	91.8 ± 2.0	0.975 ± 0.091	43.1 ± 1.9	63.8
CTM	289.3 ± 1.8	92.4 ± 1.7	1.131 ± 0.103	54.4 ± 1.8	58.5
CZP	46.2 ± 1.3	09.5 ± 0.2	0.292 ± 0.009	19.6 ± 0.7	-
CZC	125.5 ± 0.9	12.7 ± 0.6	0.468 ± 0.013	39.0 ± 1.4	-
CZE	141.8 ± 0.4	54.6 ± 0.8	0.965 ± 0.054	44.6 ± 2.1	372.2
CZM	398.8 ± 1.8	96.8 ± 1.6	1.103 ± 0.068	69.7 ± 1.6	66.3
ChTP	30.5 ± 2.0	1.9 ± 0.2	0.123 ± 0.008	11.5 ± 0.8	-
ChTC	112.0 ± 3.2	9.2 ± 1.1	0.188 ± 0.010	24.3 ± 0.6	-
ChTE	353.5 ± 5.5	32.4 ± 0.5	0.331 ± 0.013	59.5 ± 1.7	225.4
ChTB	64.5 ± 2.7	9.7 ± 0.7	0.092 ± 0.004	20.6 ± 0.8	-
ERP	43.0 ± 1.9	2.3 ± 0.6	0.112 ± 0.008	13.4 ± 0.3	-
ERC	98.0 ± 0.8	11.6 ± 0.3	0.125 ± 0.007	21.2 ± 0.7	-
ERE	88.5 ± 0.3	34.7 ± 0.8	0.115 ± 0.004	18.2 ± 0.6	-
ERB	80.5 ± 1.5	9.4 ± 0.2	0.116 ± 0.014	17.4 ± 1.1	-
JGP	45.0 ± 1.0	4.8 ± 0.1	0.100 ± 0.009	14.3 ± 1.3	-
JGC	106.0 ± 2.3	10.0 ± 0.0	0.132 ± 0.007	23.3 ± 0.7	-
JGE	296.0 ± 3.5	15.6 ± 0.9	0.311 ± 0.017	55.2 ± 2.6	-
JGB	128.5 ± 1.1	8.7 ± 0.5	0.216 ± 0.021	31.3 ± 3.1	-
RCP	34.0 ± 0.7	3.5 ± 0.2	0.110 ± 0.008	14.9 ± 0.4	-
RCC	62.4 ± 0.9	2.6 ± 0.3	0.108 ± 0.015	25.5 ± 1.5	-
RCE	406.0 ± 5.6	93.2 ± 0.7	0.532 ± 0.073	79.3 ± 2.0	31.3
RCB	547.0 ± 3.9	83.1 ± 1.0	0.373 ± 0.028	72.2 ± 2.4	65.1
BHT	-	77.8 ± 0.6	0.857 ± 0.082	64.3 ± 3.0	13.7
α -Tocopherol	-	71.4 ± 0.5	0.392 ± 0.011	72.7 ± 1.7	59.6
Gallic Acid	-	92.5 ± 0.8	0.731 ± 0.009	59.3 ± 1.6	4.4

Data are mean of triplicate determinations except IC_{50} plus ± SD, $P \leq 0.05$.

RCE, IBE, ABE and IBM (96.8, 94.3, 93.2, 92.2, 88.5 and 88.2% respectively) was stronger than BHT (77.8%) and α-tocopherol (71.4%), while the extract IEE, ABC, IBC, ABP, IBP, IEC and IEP had weaker antiradical activity than gallic acid, BHT and α- tocopherol (Table 1).

Concentration dependant DPPH assay was carried out to calculate IC_{50} values of the extracts. The results are shown in Table 1. The crude extract CTC (2.2 µg/ml), ABE (10.0 µg/ml), IBE (11.0 µg/ml), ABM (16.0 µg/ml), IEE (17.0 µg/ml), IBM (19.0 µg/ml) and IEM (20.0 µg/ml) showed results comparable with the standards (gallic acid, BHT and α - tocopherol).

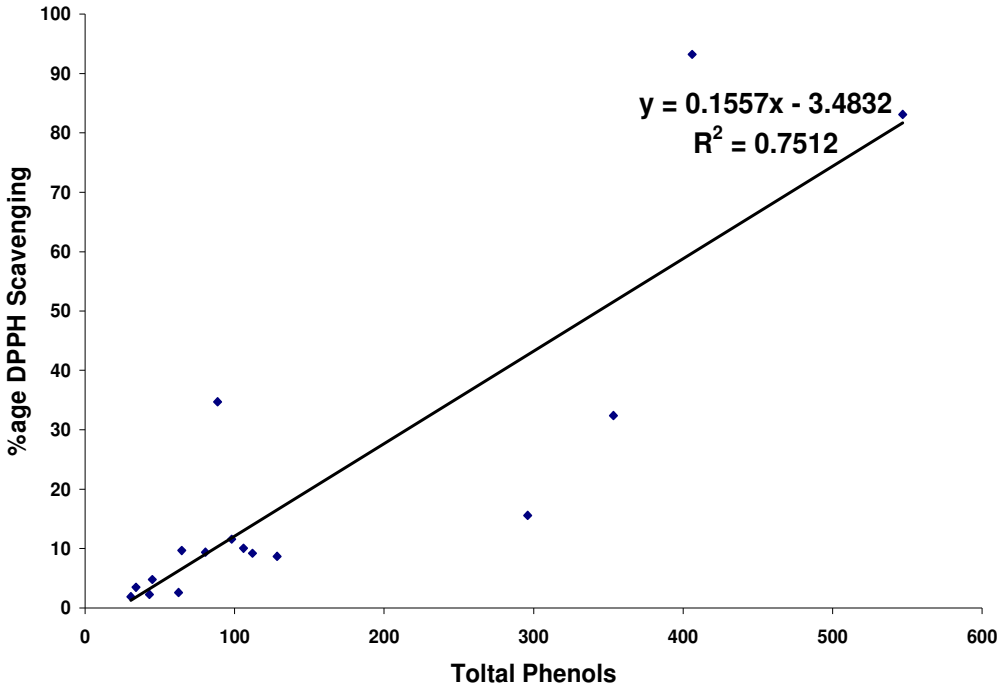

Figure 1. Correlation between total phenols and DPPH activity of extracts of Euphorbiaceae family.

Phosphomolybdate assay

The methanol and ethyl acetate extracts of *C. tamala* (CTM, CTE) and *C. zeylanicum* (CZM, CZE) showed significant results (1.131, 0.975, 1.103 and 0.965 respectively) of total antioxidant activity as compared with standards (α-tocopherol and gallic acid). The other extracts and standards were found in the order; CTC > CTP > BHT > gallic acid > RCE > IBM > IBE > ABE > IEE > ABC > α - tocopherol > IEC.

Ferric thiocyanate assay

The ethyl acetate (ABE) and methanol extracts (ABM) of *A. bidentatum* and *I. bicolor* (IBE, IBM), *I. edgeworthii* (IEE, IEM) showed 78.4, 71.9, 68.3, 68.3, 72.3 and 71.8% inhibition, respectively, in FTC method. The extracts of *C. tinctoria*, *E. royleana*, *J. gossypifolia* and *R. communis* had not shown significant activity except the extract of ethyl acetate (RCE = 79.3%) and n-butanol (RCB = 72.2%) which is in agreement with the previous reported work (Delazar et al., 2006; Upansani et al., 2003; Gorduza et al., 2000; Amic et al., 2003). The extract of *C. tamala and C. zeylanicum* also showed no significant lipid per oxidation in ferric thiocyanate method. Petroleum ether and chloroform extracts of these plants were found inactive in FTC assay. The results of RCE, ABE, IEE, ABB, IEM and IBE and IBM were comparable with BHT and α - tocopherol (Table 1).

Correlation between total phenols and antioxidant activities

The antioxidant activities of Euphorbiaceae, Lauraceae, Malavaceae and Balsaminaceae extracts as measured by DPPH, phosphomolybdate and FTC methods was found to be strongly correlated with the total phenols R^2 = 0.7512, 0.8263, 0.9332 (Euphorbiaceae), R^2 = 0.8312, 0.7127, 0.8154 (Lauraceae), R^2 = 0.9555, 0.7482, 0.9965 (Malavaceae) and R^2 = 0.9141, 0.8034, 0.8708 (Balsaminaceae), respectively (Figures 1 - 12).

Conclusion

In the past few years interest in the search of new natural antioxidants has grown because reactive oxygen species (ROS) production and oxidative stress is linked to many diseases. The use of synthetic antioxidants generally leads to problems of toxicity. In this study, antioxidant potential of nine extracts of four different plant families (Euphorbiaceae, Malvaceae, Lauraceae and Balsaminaceae) were assayed by FTC, DPPH and phosphormolybdate methods. The antioxidant activity of the extracts was also studied using linear regression analysis and found strongly correlated with total phenolic contents. These correlations suggested that polyphenols are mainly responsible for the antioxidant activity displayed by these extracts. Previous reported work on *C. tinctoria*, *J. gossypifolia and R. communis* revealed the presence of flavonoids with flavonol structure (Delazar et al., 2006;

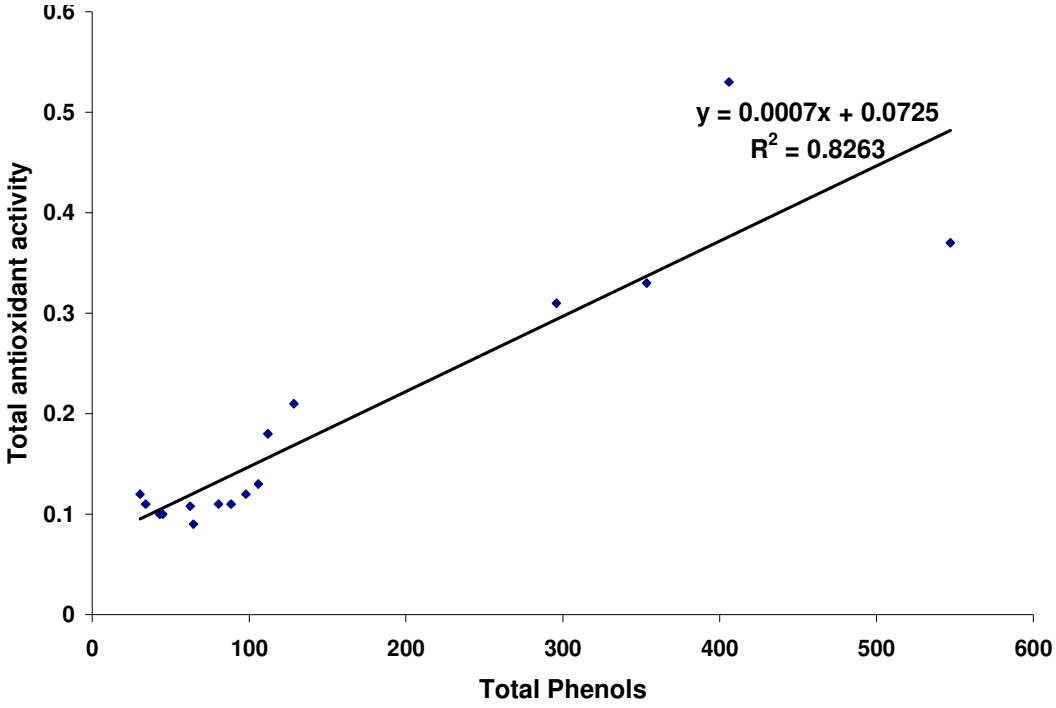

Figure 2. Correlation between total phenols and total antioxidant activity of extracts of Euphorbiaceae family.

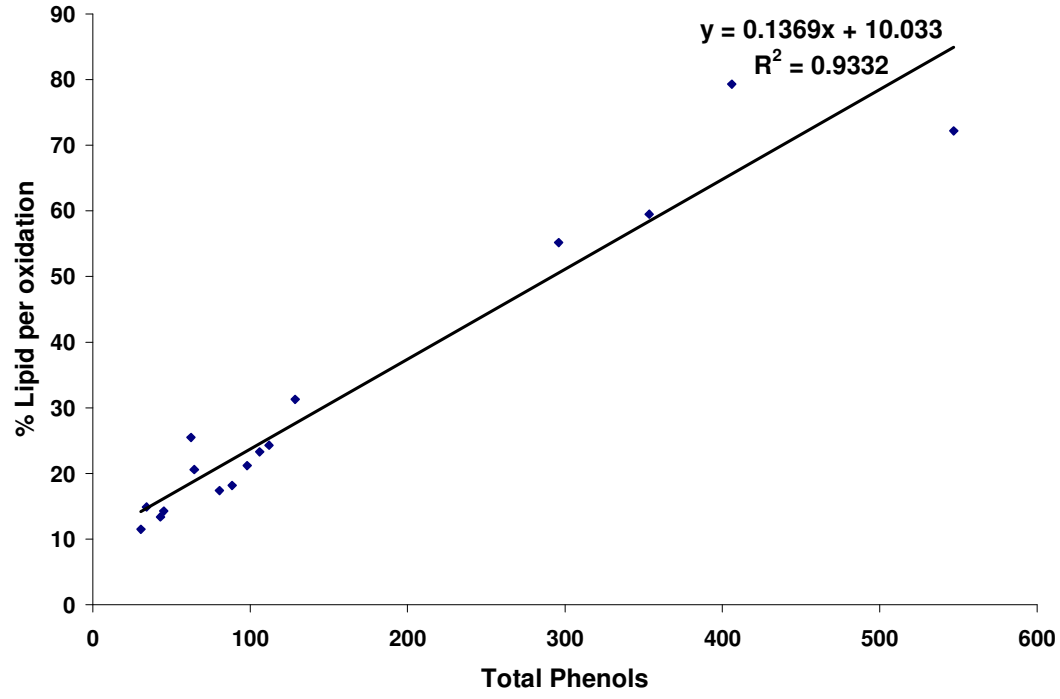

Figure 3. Co relation between total phenols and FTC method of extracts of Euphorbiaceae family.

Sankara et al., 1971; Upasani et al., 2003; Kang et al., 1985). Phytochemical studies of *C. zeylanicum* and *C. tamala* indicated three flavonoids namely quercetin, kaemferol and quercetrin (Nagendra et al., 2009), while

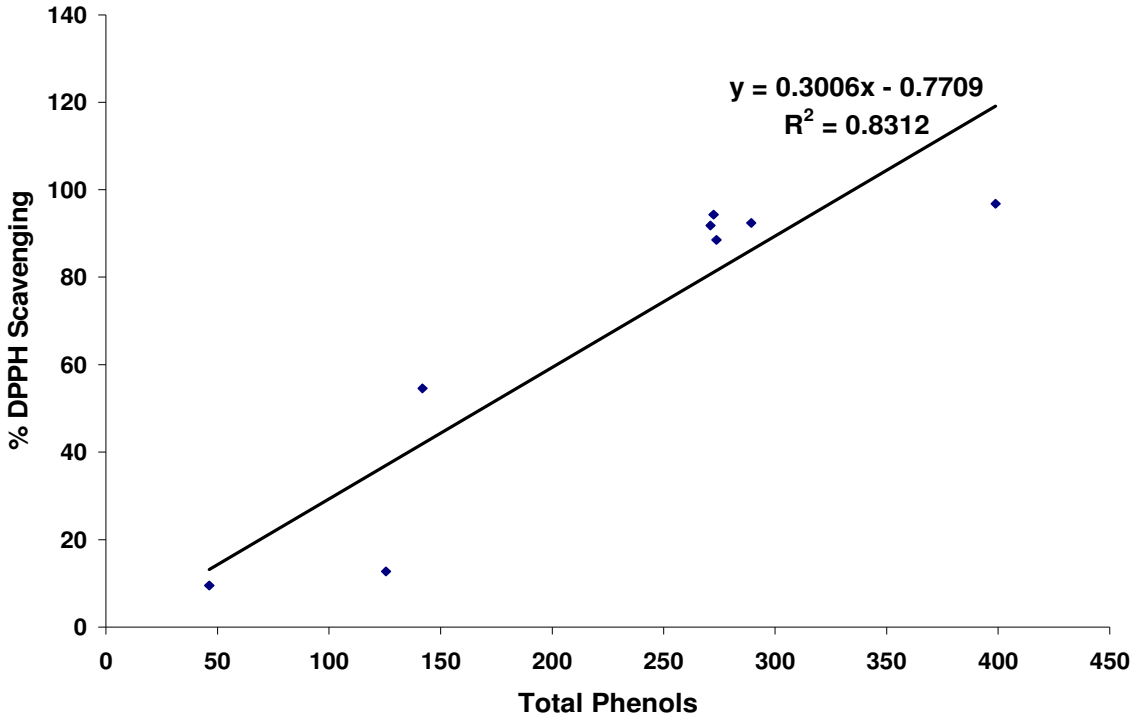

Figure 4. Correlation between total phenols and DPPH activity of extracts of Lauraceae family.

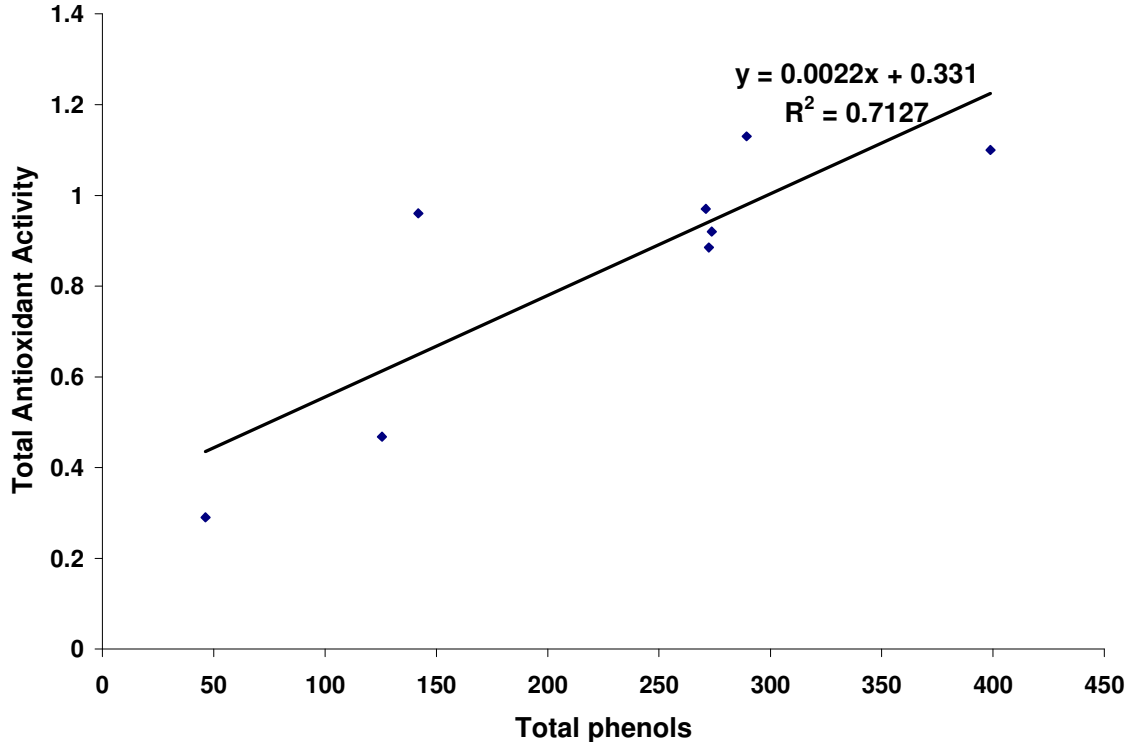

Figure 5. Correlation between total phenols and total antioxidant activity of extracts of Lauraceae family.

flavonone glycosides are reported from *I. bicolor* (Hassan and Tahir, 2005). No phytochemical studies on *A. bidentatum* and *I. edgeworthii* were found in the literature. The present results indicated that ethyl acetate and methanol

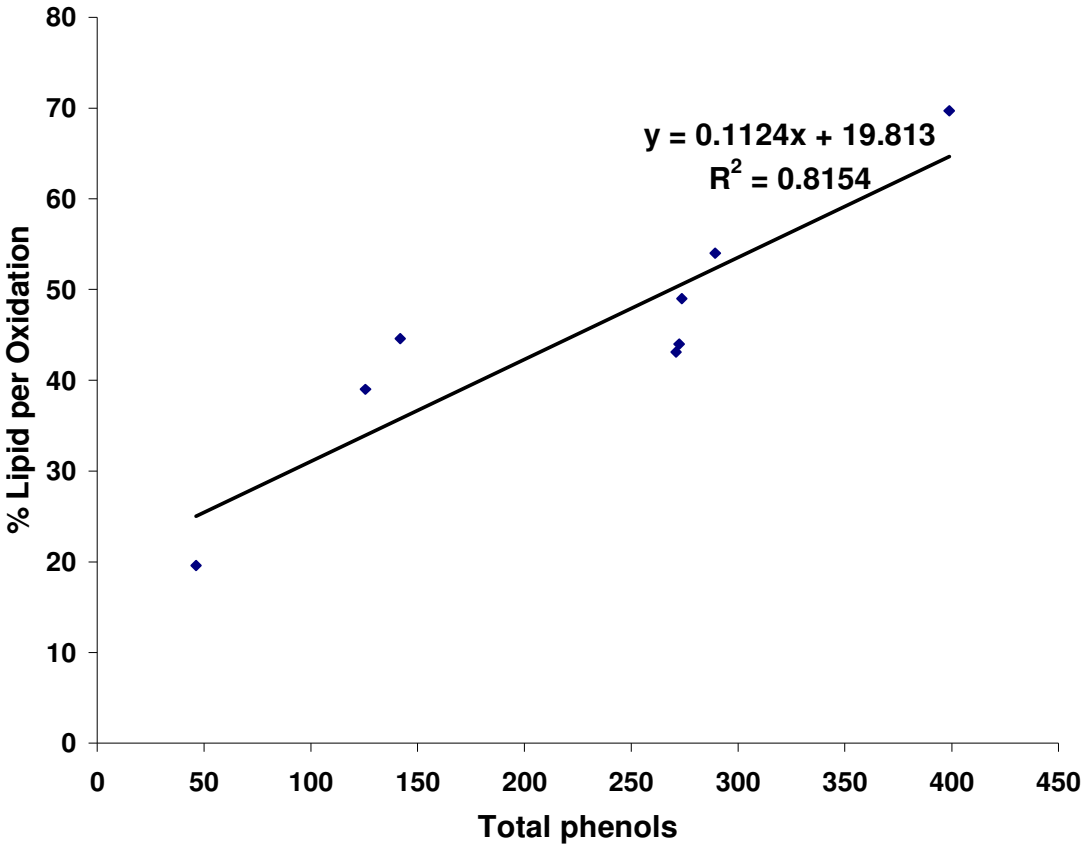

Figure 6. Correlation between total phenols and FTC method of extracts of Lauraceae family.

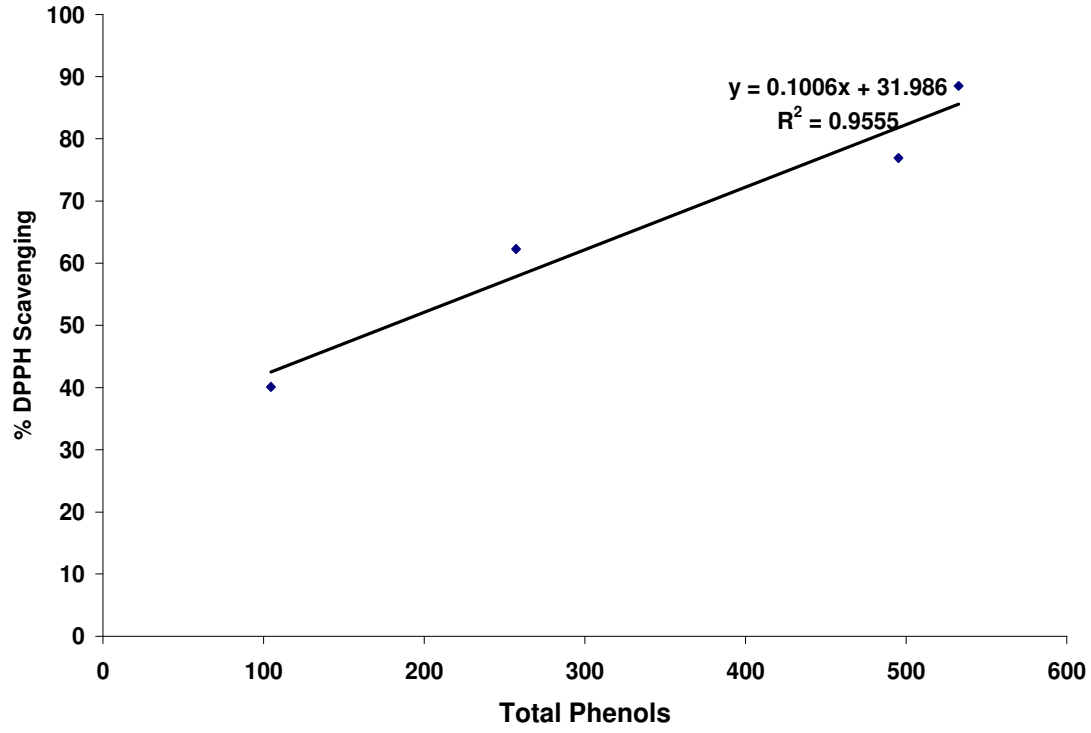

Figure 7. Correlation between total phenols and DPPH activity of extracts of Malvaceae family.

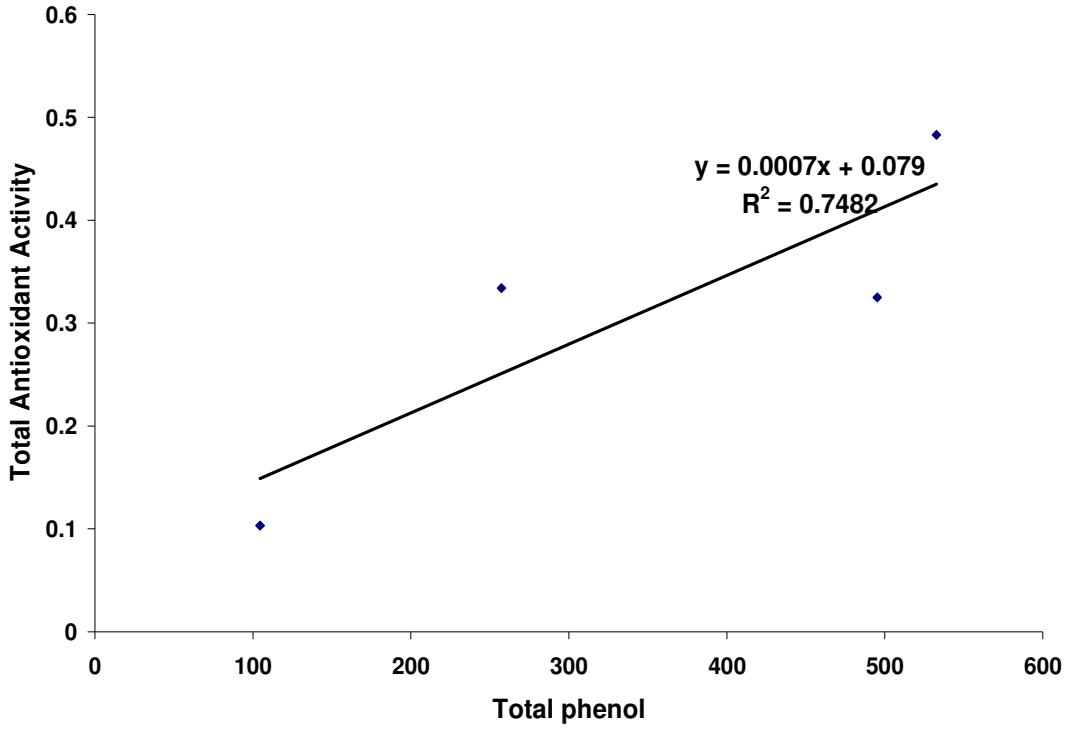

Figure 8. Correlation between total phenols and total antioxidant activity of extracts of Malvaceae family.

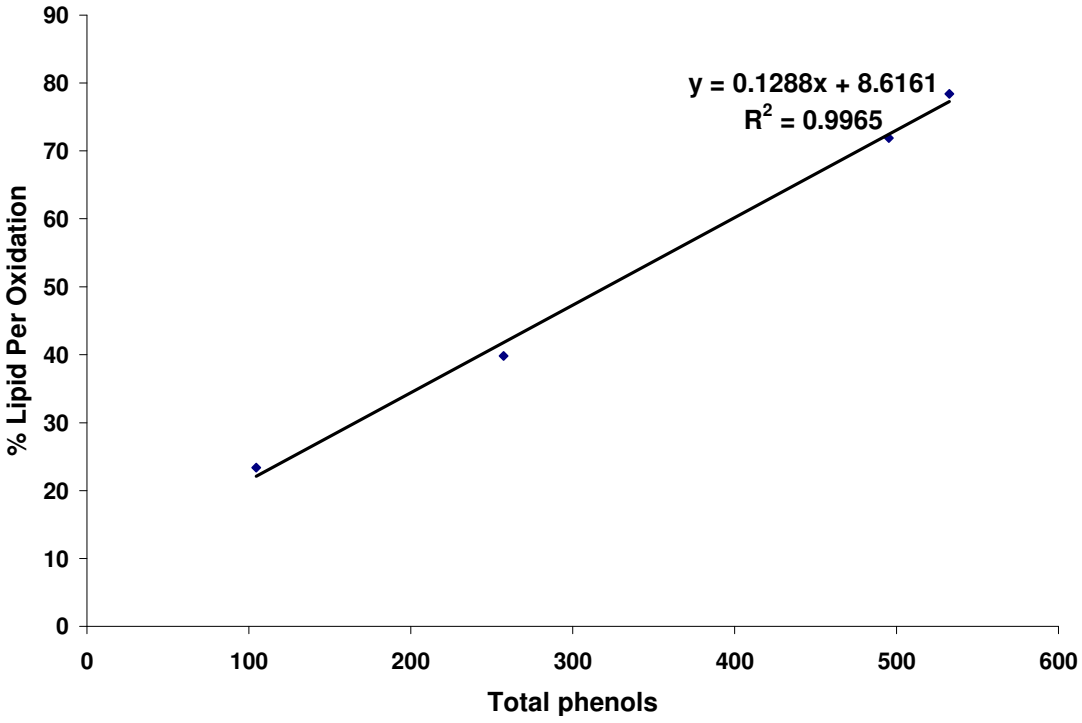

Figure 9. Correlation between total phenols FTC method of extracts of Malvaceae family.

extracts of *A. bidentatum* and *I. edgeworthii* are rich in polyphenols which is the first report, therefore, it is suggested that these extracts can provide potential natural antioxidants. More research is still needed to determine both composition and structure of phenolics present in the active extracts.

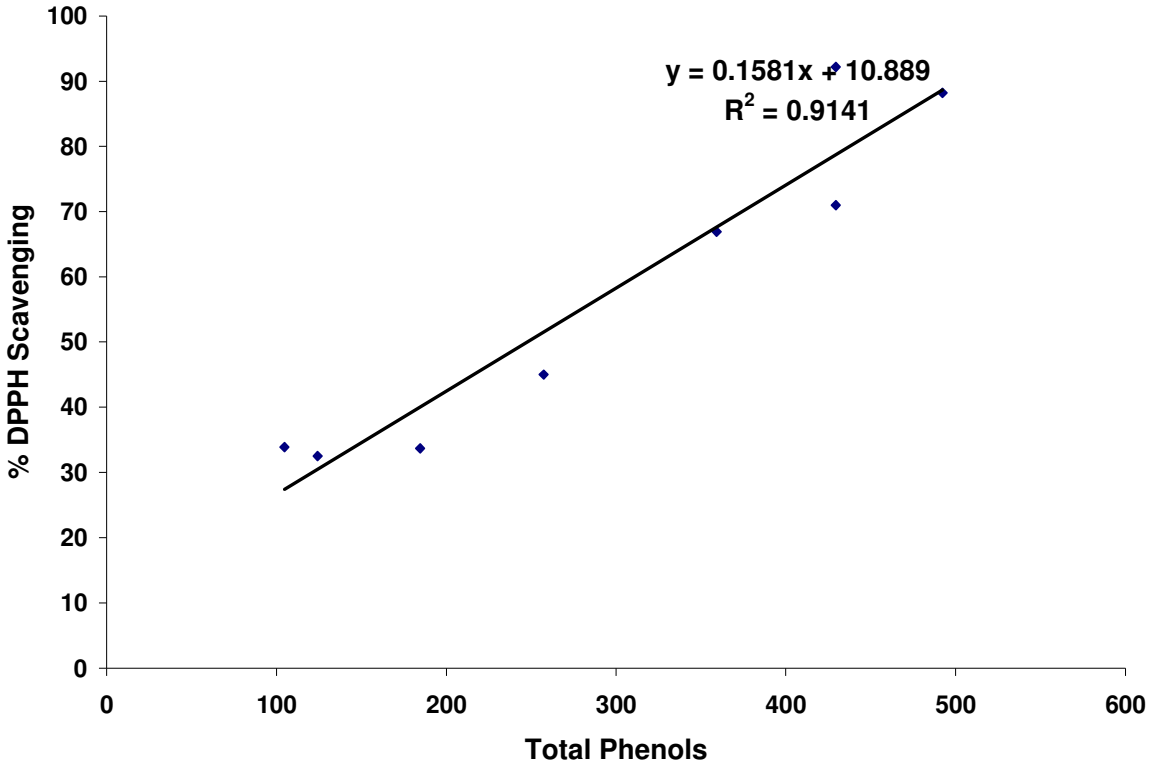

Figure 10. Correlation between total phenols and DPPH of extracts of Balsaminaceae family.

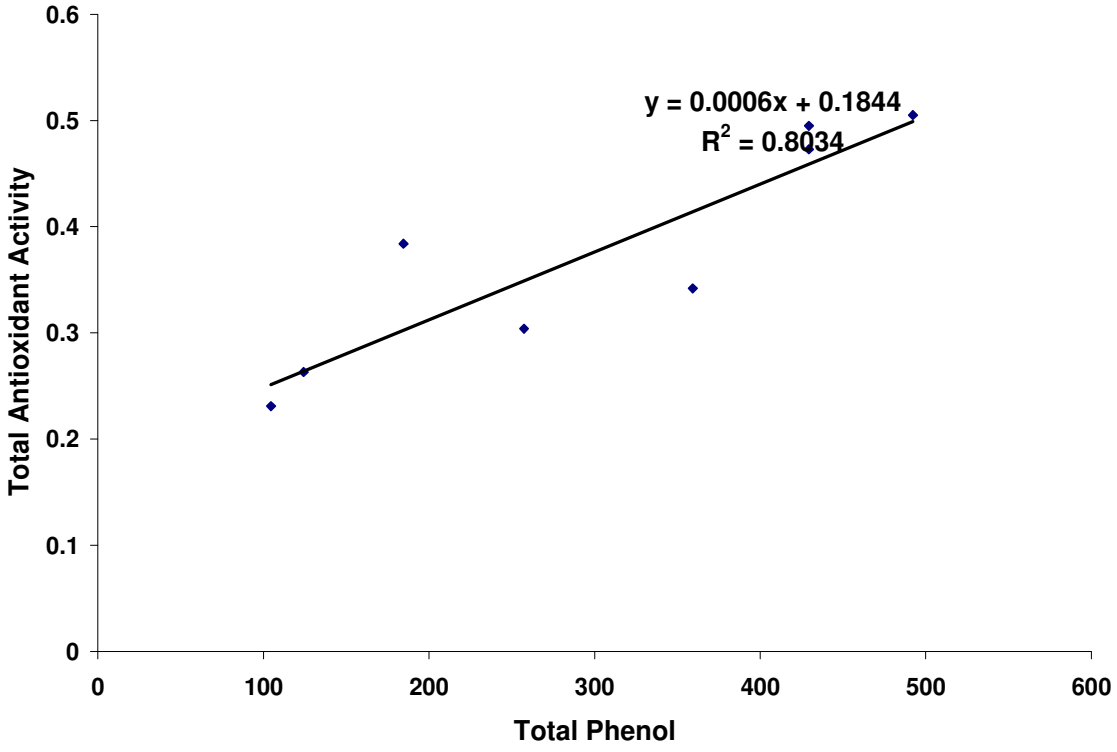

Figure 11. Correlation between total phenols and total antioxidant activity of extracts of Balsaminaceae family.

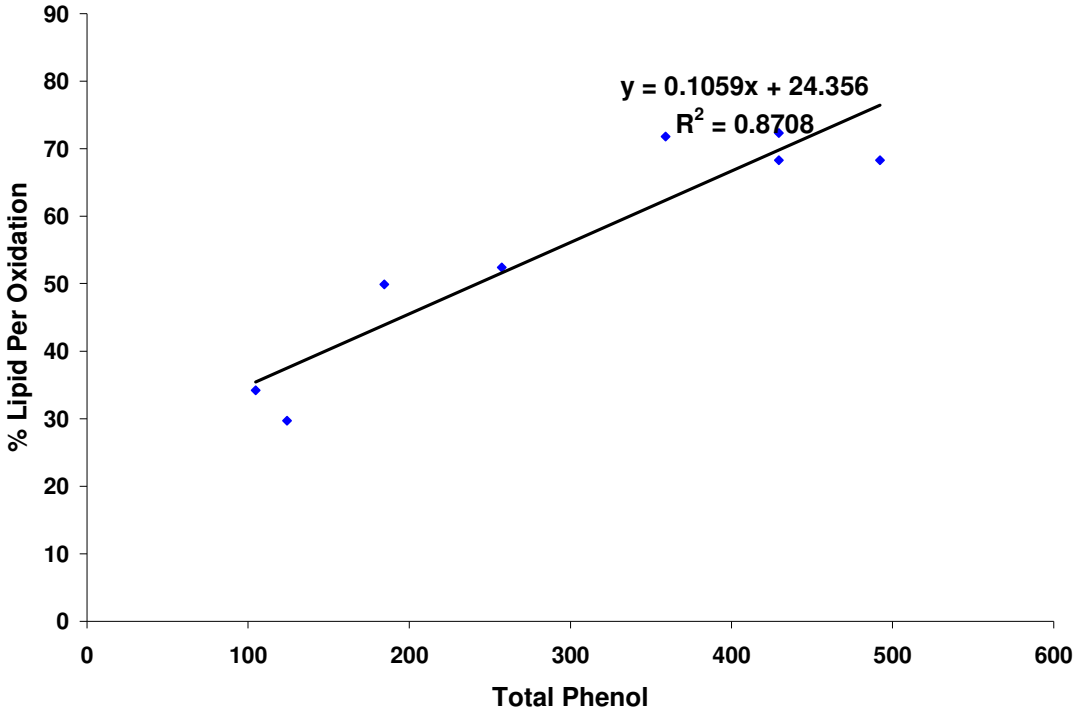

Figure 12. Correlation between total phenols and FTC method of extracts of Balsaminaceae family.

Abbreviations:

BP: *A. bidentatum* in pet. ether, **ABC**: *A. bidentatum* in chloroform, **ABE**: *A. bidentatum* in ethyl acetate, **ABM**: *A. bidentatum* in methanol, **IBP**: *I. bicolor* in pet. ether, **IBC**: *I. bicolor* in chloroform, **BE**: *I. bicolor* in ethyl acetate, **IBM**: *I. bicolor* in methanol, **IEP**: *I. edgeworthii* in pet. ether, **IEC**: *I. edgeworthii* in chloroform, **IEE**: *I. edgeworthii* in ethyl acetate, **IEM**: *I. edgeworthii* in methanol, **CTP**: *C. tamala* pet. ether, **CTC**: *C. tamala* in chloroform, **CTE**: *C. tamala* in ethyl acetate, **CTM**: *C. tamala* in methanol, **CZP**: *C. zeylanicum* in pet. ether, **CZC**: *C. zeylanicum* chloroform, **CZE**: *C. zeylanicum* in ethyl acetate, **CZM**: *C. zeylanicum* in methanol extract, **ChTP**: *C. tinctoria* in pet. ether, **ChTC**: *C. tinctoria* chloroform, **ChTE**: *C. tinctoria* in ethyl acetate, **ChTB**: *C. tinctoria* in n-butanol extract, **ERP**: *E. royleana* in pet. ether, **ERC**: *E. royleana* in chloroform, **ERE**: *E. royleana* in ethyl acetate, **ERB**: *E. royleana* in n-butanol, **JGP**: *J. gossypifolia* in pet. ether, **JGC**: *J. gossypifolia* chloroform, **JGE**: *J. gossypifolia* in ethyl acetate, **JGB**: *J. gossypifolia* in n-butanol, **RCP**: *R. communis* in pet. ether, **RCC**: *R. communis* chloroform, **RCE**: *R. communis* in ethyl acetate, **RCB** : *R. communis* in n-butanol, - = no significant results.

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