THE ANTIOXIDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF LEAVES AND BARK OF BACCAUREA RAMIFLORA (LOUR.) HAVE BEEN MEASURED BY IN VITRO CHEMICAL ANALYSES.

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Abstract

In this work we have find out the antioxidants activity of the ethyl acetate, methanolic and aquaous extract of leaves and bark of Baccaurea ramiflora (Lour.). The antioxidant activity of the extracts was measured by in vitro chemical analyses involving the assays of (1) Au nanoparticle formation potential (2) 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (3) ferric ion reducing power and (4) ferrous ion chelating activity. A simpler method has been created based on Au nanoparticles formation to assess the antioxidant activity of any plant extract. It was for the assessment of the antioxidant activity of all the extract of leaves and bark of Baccaurea ramiflora (Lour.). In all the assays, methanolic extract of leaves of Baccaurea ramiflora (MEBRL) and methanolic extract of bark of Baccaurea ramiflora (MEBRB) showed significantly greater activity over other extracts. This work provides a scientific support for the high antioxidant activity of this plant and thus it may find potential applications in the treatment of the diseases caused by ROS.

KEYWORDS

Antioxidants, Baccaurea ramiflora (Lour.), DPPH, Gold Nanoparticles(AuNps), MEBRL, MEBRB

1. INTRODUCTION

Free radicals, e.g. various reactive oxygen species (ROS), are allways produced during Free radicals, e.g. various reactive oxygen species (ROS), are allways produced during cellular metabolism in living systems and them because a number of oxidative stresses related disorders in human beings, such as atherosclerosis, ageing, cancer and cardiovascular diseases [1-4]. Natural antioxidants put a wall against the damages of the cellular organelles caused by free radical induced oxidative stress [5]. Apart from this, antioxidants also control the nutritional quality of the foods by reducing the nutritional loss and preventing the formation of harmful substances during the storage of the food. Various studies also suggest that a predominantly plant based diet reduces the risk of free radical induced diseases [6]. So, the scientist found it challenging to screen the plants on the basis of their antioxidant activity. Large numbers of medicinal plants have been registred for their antioxidant activities and the results proves that either their raw extracts or their individual chemical constituents are more effective antioxidants (in vitro) than the synthetic antioxidants, e.g. butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) or vitamin E [7-9]. Moreover, these synthetic antioxidants, e.g. butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) etc., may have carcinogenic and other harmful effects on the lungs and livers [10-12]. So, scientists are continuously involved to find out naturally occurring potential and non-toxic

antioxidants, which could prevent this free radical related disorders in human beings and also can replace the harmful synthetic antioxidants[10-12].The Indian medicinal plants which are traditionally used as an integral part of Indian Ayurveda, can be the potential sources for various naturally occurring non-toxic antioxidants.

In our experiments we have found out the antioxidants activity of the ethyl acetate, methanolic and aqua extract of leaves and bark of Baccaurea ramiflora (Lour.). Baccaurea ramiflora (Lour.), (family: Euphorbiaceae) is a tree that grows slowly and evergreen could reach up to the height of 25 m, has spreading crown and thin bark. The fruit of B. ramiflora is yellow to red in color. We can find this tree in the Southeast Asian region and it also grows wild as well as under cultivation in Nepal, India, Myanmar, South China, Indo-China, Thailand, the Andaman Islands and Peninsular Malaysia. It mainly grows in evergreen forests. Latkan or Bhubi (Bengali), Leteku (Hindi), Mafai (Thai) and Burmese grape (English)[13-14] are the common names of this tree. In the traditional Chinese Dai medicine, the whole plant of B. ramiflora is used as an antiphlogistic and anodyne against rheumatoid arthritis, cellulitis, and abscesses and to treat injuries [15]. In Northern Thailand the plant is also used as medicine by hill-tribes [16]. Young leaves of B. ramiflora are used as vegetable, flavoring agent with curries and minced meat in Bangladesh [17]. In India, fresh bark is chewed or juice is used orally for constipation. That is why; we have chosen the leaf and bark of B. ramiflora as our research work.

The objective of the present study is to explore the antioxidant activity of the ethyl acetate, methanolic and aqua extract of leaves and bark of Baccaurea ramiflora (Lour.). Baccaurea ramiflora (Lour.), (family: Euphorbiaceae). The leaves and bark of this plant is selected because it is easily available throughout the year and the collection of the leaves and bark do not destroy the parent plant. Here we have used four different in vitro chemical assays (1) gold (Au) nanoparticle formation potential assay (2)1,1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay (3) ferric ion reducing antioxidant power assay and (4) ferrous ion chelating activity assay. We have found in our work a detailed assay of the antioxidant activity of two parts of different extracts of this plant. We also used a simple method to assess the antioxidant activity of any plant extract by its ability to form Au nanoparticles and this method is exploited to estimate the antioxidant activity of the ethyl acetate, methanolic and aqua extract of leaves and bark of Baccaurea ramiflora (Lour.)The total polyphenol and flavonoid contents of the ethyl acetate, methanolic and aqua extract of leaves and bark of Baccaurea ramiflora (Lour.)were also measured according to the standard methods[18-21] and these values were correlated to the antioxidant activity of these extracts. cellular metabolism in living systems and them because a number of oxidative stresses related disorders in human beings, such as atherosclerosis, ageing, cancer and cardiovascular diseases [1-4]. Natural antioxidants put a wall against the damages of the cellular organelles caused by free radical induced oxidative stress [5]. Apart from this, antioxidants also control the nutritional quality of the foods by reducing the nutritional loss and preventing the formation of harmful substances during the storage of the food. Various studies also suggest that a predominantly plant based diet reduces the risk of free radical induced diseases [6]. So, the scientist found it challenging to screen the plants on the basis of their antioxidant activity. Large numbers of medicinal plants have been registred for their antioxidant activities and the results proves that either their raw extracts or their individual chemical constituents are more effective antioxidants (in vitro) than the synthetic antioxidants, e.g. butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) or vitamin E [7-9]. Moreover, these synthetic antioxidants, e.g. butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) etc., may have carcinogenic and other harmful effects on the lungs and livers [10-12]. So, scientists are continuously involved to find out naturally occurring potential and non-toxic antioxidants, which could prevent this free radical related disorders in human beings and also can replace the harmful synthetic antioxidants[10-

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2. MATERIALS AND METHODS

2.1.Chemicals

Folin–Ciocalteu reagent, gallic acid, quercetin, DPPH, trichloroacetic acid, ascorbic acid, methanol and ethyl acetate have been purchased from Sigma Aldrich. All other chemicals used for the study were of analytical grade and obtained from MERK, India. Double distilled water has been used for all the analyses. Chloroauric acid (HAuCl4) (Sigma Aldrich,) have been used as the source of Au (III) ions required for the synthesis of Au nanoparticles.

2.2. Collection and preparation for stock

At first**,** Leaves and bark of Baccaurea ramiflora (Lour.) have been collected from local area of Alipuarduar, West Bengal, India. Then these are washed with distilled water and dried under shade. Next, the dry leaves and the barks have been grinded and stored into an air tight container for further use. All chemicals/reagents we have used in the study were of analytical grade and have been purchased from Merck and Sigma-Aldrich Chemical Company. All reagents and

spectroscopic grade solvents have been used as we have received from commercial sources without further purification. Aqueous medium experiments have been done in deionized water.

2.3. Preparation of Leaf and Bark Extract

The leaves and barks extract have been prepared using the dried leaves and bark of Baccaurea ramiflora (Lour.). About 100 gm of each dried leaves and bark have been taken into a 1 litre round bottom flask and about 300ml of deionized water, 300ml of methanol and 300ml of ethyl acetate have been added and refluxed for 8 hr. The insoluble materials have been filtered off. The filtrate was then made to freeze-dry and a semi-solid was obtained from it. The semi-solid mass was stored at 4 °C for further use.

3. RESULTS AND DISCUSSION

3.1. Estimation of Total Phenols and Flavonoid Content

The method we have used for the determination of total phenols using Folin Ciocalteu reagent and gallic acid as standard using the reported procedure. Total phenol values are expressed as gallic acid equivalents per gram of dry mass. The aluminum chloride colorimetric method was used for flavonoid content determination of leaves and bark of Baccaurea ramiflora (Lour.) extract quercetin (standard) solutions by following the reported procedure [22]. The total flavonoid content in these extracts was expressed in terms of milligram of quercetin equivalent per gram of dry mass. Interestingly, MEBRL and MEBRB have higher polyphenol flavonoid content.

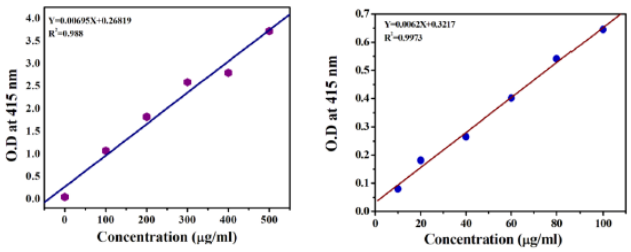

Figure 1: Calibration curve of (a) polyphenol content (Gallic Acid) and (b) flavonoid content (Quercetin)

Table 1: Polyphenol and Flavonoid content of the all Extracts

sample	EEBRL	MEBRL	AEBRL	EERBB	MEBRB	AEBRB
Polyphenol content(mg GAE g^{-1})	37.33±0.27	58.69±0.14	48.86±0.15	25.63±0.12	49.62±0.18	38.93±0.22
Flavonoid content(mg QE g^{-1})	18.14±0.16	33.32±0.21	4.14± 0.28	12.83±0.22	27.33± 0.26	2.40± 0.25

3.2. Au Nanoparticles Formation Potential based Antioxidant Assays

The antioxidant activity of the extracts has been measured from their ability to generate and stabilize Au nanoparticles. Here we have simplified the methods of Scampiccho et al. and Wang et al. [23-24]. Sample solutions of different concentrations (100µg ml^{-1} to 800µg ml^{-1}) have been prepared separately by dissolving the appropriate quantities of gummy masses obtained from the

extracts in distilled water. To 10ml of this sample solution, 250µL of 0.1 M aq. $HAuCl_4$ solution have been added followed by continuous stirring and heating at 45°C for 10 minutes; whereby a pink coloration was observed which is the indication of the onset of formation of Au nanoparticles. The progress of the reaction was monitored by measuring the absorbance of the solution in the range of 500-700 nm at regular interval of time. For the calibration of the standard, aqueous extract of amla (AEA) of the same concentration range have been used. The results are expressed as the milligram of AEA per gram of dry mass of the sample extracts.

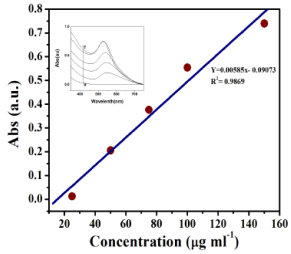

Figure 2: Calibration curve of the standard compound of aqueous extract of amla (AEA).

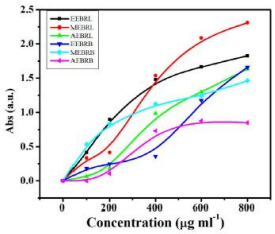

Figure 3: Plotting of absorbance maxima vs. different concentrations. of Au nanoparticle solutions formed after a 10-min reaction at 45°C.

Table 2: AuNps formation potential of the all Extracts

Sample	AuNps formation potential (mg AEA/gm)
EEBRL	449.475±6.350
MEBRL	596.329±7.456
AEBRL	379.286±5.382
EEBRB	341.899±6.341
MEBRB	479.023±5.842
AEBRB	238.710±4.564

3.3. DPPH Radical Scavenging Activity

The free radical scavenging activities of the all extracts have been evaluated through their ability to quench the synthetic DPPH radical. DPPH has been used to determine free radical scavenging activities of the all extracts by using standard method[25]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The methodology involves reaction of specific compound or extract with DPPH in methanol solution. In the presence of hydrogen donors, DPPH is reduced and a free radical is formed from the scavenger. The reaction of DPPH is monitored by measuring the decrease of the absorbance of its radical at 517 nm. Upon reduction of this radical by an antioxidant, the absorbance at 517 nm disappears.

IC_{50} value of MEBRL was 68.32µg ml^{-1} while that of MEBRB is 47.61µg ml^{-1}. Both of these two values are comparable with the IC_{50} value of the standard compound, Gallic acid; which has been found to be 15.98µg ml^{-1}. Thus MEBRL and MEBRB showed higher radical scavenging activity than that of other extracts. Moreover, both of these two values are significantly lower than that of some Indian green leafy vegetables [26].

Table 3: IC_{50} value of all the extracts and standard compound

Sample	EEBRL	MEBRL	AEBRL	EEBRB	MEBRB	AEBRB	Gallic Acid
DPPH Scavenging Ability(IC_{50}, µg/ml)	37.38±2.68	68.32±2.28	41.21±1.63	29.06±2.03	47.61±2.21	43.19±2.74	15.98±3.65

The higher activity of MEBRL and MEBRB was probably due to its higher polyphenol content and also due to the better solubility of its polyphenol constituents in methanol. The difference in antioxidant activities of these extracts can be attributed to the presence of different types of flavonoids compounds for showing antioxidant activity [27]

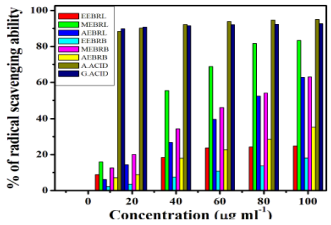

Figure 4: DPPH radical scavenging activities of all extracts, ascorbic acid and gallic acid (standard) solutions at different concentrations. All data are reported as mean± S.D. (n=3).

3.4. Ferric Reducing Antioxidant Power Assay

Reducing power of a compound is also a supporting feature for its antioxidant activity. Reducing power characteristics of the all extracts and ascorbic acid (standard compound) are given in Fig.5. The concentration dependent reducing power followed the order of: ascorbic acid > MEBRB > other. At lower concentration region, MEBRL showed slightly higher reducing power, but as a whole MEBRB had higher reducing activity. This may be due to the higher polyphenol content of this extract. Because being good electron donor, phenolic compounds have the ability to convert Fe^{3+} to Fe^{2+} and hence show higher reducing activity.

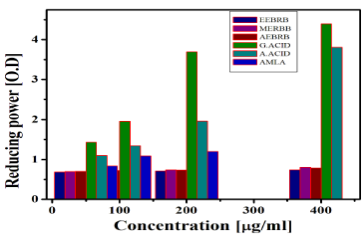

Figure 5: Reducing power of all extracts, galic acid and ascorbic acid (standard) solutions at different concentrations (0 to 400µg ml^{-1}).

Table 4: EC_{50} value of all extracts

Sample	EEBRL	MEBRL	AEBRL	EEBRB	MEBRB	AEBRB
FRAP (EC_{50}, µg/ml)	469.222± 8.22	1017.505± 8.76	712.281± 8.32	1369.497± 7.01	1512.424± 7.94	748.892± 5.33

3.5. Ferrous Ion Chelating Activity Assay

Fe^{2+} ions are abundant in foods and it is a well known and effective pro-oxidant which catalyses various oxidation reactions in biological systems [28]. Ferrous ion chelating activity assay of any extract or any compound actually measures the capacity of that extract or that compound to bind the Fe^{2+} ion. 1, 10- phenanthroline can quantitatively bind with Fe^{2+} to form a stable complex which is colored red and has the absorbance maximum at 510 nm. The absorbance of this complex at 510 nm steadily decreases with the increase of the chelating activity of chelating agents.

In our case, a concentration dependant chelating activity was noticed for all the extracts and also for the EDTA (standard) solutions as shown in fig.6. The chelating activity of EDTA is significantly higher than that of all the extracts. T-Test revealed that chelating activity of MEBRB is significantly higher than that of others (P=0.00).

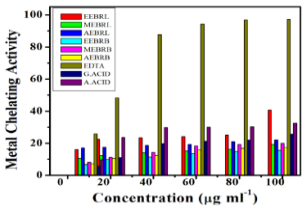

Figure 6: Fe^{2+}ion chelating activities of all extracts, galic acid, ascorbic acid and EDTA (standard) solutions at different concentrations (10-100 µg ml^{-1}). All data are reported as mean± S.D. (n=3).

4. CONCLUSIONS

In all the assay systems, it was found that methanolic extract of leaves (MEBRL) and bark of Baccaurea ramiflora(MEBRB) showed higher antioxidant activity over other extracts. But both these high polyphenol and flavonoid contents than those of others. So in addition to polyphenol and flavonoids, there must be some other non-phenolic components (may be reducing sugars) present in these extracts which also are responsible for its antioxidant activity. Our observations may enhance the potential application of methanolic extract of leaves and bark of Baccaurea ramiflora(L.)as antioxidant in various pharmaceutical products. Moreover, this extract is always more bio-friendly than any other organic solvent extracts. So MEBRL and MEBRB may be a well substitute of other and can be explored for its applications in the prevention of free radical related diseases. Moreover, the high antioxidant activity of these plant extracts may be explored in controlling the nutritional quality of the foods by reducing the nutritional loss and preventing the formation of harmful substances during the storage of the food.

REFERENCES

[1] B.A. Freeman, (1984) Biological sites and mechanism of free radical production. In: Free radicals in molecular biology aging and disease, New York, USA:Raven Press, pp. 43–52.

[2] M. R. Moein, S. Moein, & S. Ahmadizadeh, (2008) Radical scavenging and reducing power of Salvia mirzayanii subfractions, Molecules, vol. 13, pp. 2804–2813.
[3] A. Singh, (1989) Physicochemical and physiological aspects. In: CRC handbook offree radicals and antioxidants in biomedicine Boca Raton,Florida, USA: CRC Press Inc, vol. 1, pp. 123–126.
[4] G. L. Squadrito, & W. A. Pryor, (1998) Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite, and carbon dioxide, Free Radical Biology and Medicine, vol. 25, pp. 392–403.
[5] Y. Quinming, P. Xianhui, K. Weibao, Y. Hong, S. Yidan, & Li. Zhang, (2010) Antioxidant activities of malt extract from barley (Hordeum vulgare L.) towards various oxidative stress in-vitro and in-vivo, Food Chemistry, vol. 118, pp. 84–89.
[6] S. L. Halvorsen, K. Holte, M. C. W. Muhrstand, I. Barikmo, E. Hvattum, & S.F. Remberg, (2002) Systematic screening of total antioxidants in dietary plants, Journal of Nutrition, vol. 132, pp. 461–471.
[7] M. H.Gordon, & X. C.Weng, (1992) Antioxidant properties of extracts from Tanshen (Salvia miltiorrhiza Bunge), Food Chemistry, vol. 44, pp. 119–122.
[8] L. W. Gu, & X. C. Weng, (2001) Antioxidant activity and components of Salvia plebeia R. Br. – a Chinese herb, Food Chemistry, vol. 73, pp. 299–305.
[9] Y. H. Pyo, & T. C. Lee, L. Logendrac, R. T. Rosen, (2004) Antioxidant activity and phenolic compounds of Swiss chard (Beta vulgaris subspecies Cycla) extracts, Food Chemistry, vol. pp. 85, 19–26.
[10] A. L. Branen, (1975) Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene, Journal American Oil Chemists' Society, vol. 52, pp. 59–63.
[11] H. C. Grice, (1986) Safety evaluation of butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract, Food and Chemical Toxicology, vol. 24, pp. 1127–1130.
[12] H. P. Wichi, (1988) Enhanced tumor development by butylated hydroxyanisole (BHA) from the perspective of effect on forestomach and oesophageal squamous epithelium. Food and Chemical Toxicology, vol. 26, pp. 717–723.
[13] B. Khan,(2008) Encyclopedia of Flora and Fauna of Bangladesh. Asiatic Society of Bangladesh, Dhaka, Edition1, Vol. 7, pp. 392-393.
[14] SV. Hoang, B. Pieter, & PJA. Keβler, (2008) Uses and Conservation of Plant Species in a National Park- A Case Study of Ben En, Vietnam. Economic Botany, vol. 62(4) pp. 574–593.
[15] YF. Lin, Z. Yi, & YH. Zhao,(2003) Chinese Dai Medicine Colorful Illustrations. Yunnan Nationality Press, First Edition.
[16] XW.Yang, JS.Wang, YL.Ma, HT. Xiao, YQ. Zuo, H. Lin, HP.He, L.Li, & XJ. Hao, (2007) Bioactive Phenols from the Leaves of Baccaurea ramiflora. Planta Medica, vol. 73 pp. 1415-1417.
[17] SMR. Hasan, MM.Hossain, R. Akter, M. Jamila, MEH. Mazumder, & S. Rahman, (2009) DPPH free radical scavenging activity of some Bangladeshi medicinal plants, Journal of Medicinal Plants Research, vol. 3(11) pp. 875-879.
[18] C. Chang, M. Yang, H. Wen, & J. Chern, (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods, Journal of Food and Drug Analysis, vol. 10, pp. 178–182.
[19] S. Mcdonald, P. D. Prenzler, M. Autolovich, & K. Robards,(2001) Phenolic content and antioxidant activity of olive extracts, Food Chemistry, vol. 73, pp. 73–84.
[20] R. A. Laskar, I. Sk, N. Roy, & N. A.Begum, (2010) Antioxidant activity of Indian propolis and its chemical constituents, Food Chemistry, vol. 122, pp. 233–237.
[21] N.Roy, S.Mondal, R. A. Laskar, S. Basu, D. Mandal, & N. A. Begum, (2010) Biogenic synthesis of Au and Ag nanoparticles by Indian propolis and its constituents, Colloids and Surfaces B: Biointerfaces, vol.76, pp.317–325.
[22] C. Chang, M. Yang, H. Wen, & J. Chern, Estimation of total flavonoid content in propolis by two complementary colorimetric methods, J. Food and Drug Analysis, vol.10, pp. 178-182.
[23] M. Scampicchio, J. Wang, A.J. Blasco, A.S. Arribas, S. Mannino, & A. Escarpa,(2006) Nanoparticle-based assays of antioxidant activity, Analytical Chem, vol. 78, pp. 2060-2063.
[24] J. Wang, N. Zhou, Z. Zhu, J. Huang, & G. Li, (2007) Detection of flavonoids and assay for their antioxidant activity based on enlargement of gold nanoparticles, Analytical and Bioanalytical Chemistry, vol. 388, pp.1199-1205.
[25] I. I. Koleva, T. A. van Beek, J. P. H. Linssen, A. De Groot, & L. N. Evstatieva, (2002) Screening of plant extracts for antioxidant activity:A comparative study on three testing methods, Phytochemical Analysis, vol.13, pp.8-17.

[26] S. Gupta, & J. Prakash,(2009) Studies on Indian green leafy vegetables for their antioxidant activity, Plant foods and Human Nutrition, vol. 64 pp. 39-45.
[27] D. Zielinska, D. Szawara-Nowak, A. Ornatowska, & W. Wiczkowski,(2007) Use of Cyclic Voltammetry, Photochemiluminescence, and Spectrophotometric Methods for the Measurement of the Antioxidant Capacity of Buckwheat Sprouts, J. Agric. Food Chem, vol. 55, pp.9891–9898.
[28] M.Y. Shon, S.D. Choi, G.G. Kahng, S.H. Nam, & N.J. Sung,(2004) Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions, Food and Chemical Toxicology, vol.42 659–666.

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