International Journal on Emerging Technologies **10**(2): 243-248(2019)

ISSN No. (Print) : 0975-8364
ISSN No. (Online) : 2249-3255

Evaluation of Polyphenolic Content and Anti-oxidant Potential of Poly-herbal Formulation

Rajesh Saini[1], Awadhesh Kumar Mishra[1], Sunil Kumar Mishra[2] and Kavindra NathTiwari[1]*
[1]*Department of Botany, MMV, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India*
[2]*Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India*

(Corresponding author: Dr. Kavindra Nath Tiwari)
(Received 17 April 2019, Revised 29 July 2019 Accepted 3 August 2019)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: In the present study a new poly-herbal formulation was prepared using fresh leaves of *Lantana camara, Magnolia obovata, Albizzia lebbeck*; stem bark of *Clitoria ternatia,Cinnamomum cassia* and dry fruits of *Coriander sativum*. Hydro-alcoholic (50:50) extract was prepared using soxhlet apparatus. Estimation of total phenol, tannin, flavonoid and flavonol content in the extract was performed. The extract contains phenol 2.37 mg/gm gallic acid equivalent (GAE) and tannin 0.96 mg/gm tannic acid equivalent (TAE). The flavanoid content was 8.20 mg/gm rutin equivalent (RE), and flavonol content was 6.98 mg/gm quercetrin equivalent (QE) in the extract. The total antioxidant activity of poly-herbal formulation extract was 0.217 absorption unit (au). Antioxidant estimation by 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical scavenging activity was measured and result revealed that extract caused 50% inhibition at 104.74 µg/ml. Similarly, hydrogen peroxide scavenging activity was measured and EC_{50} of the extract was 46.89 µg/ml. Reducing power of the extract was found to be 0.454±0.014 µg/ml. The poly herbal formulation can be used as good source of natural anti-oxidant for health care and reducing the onset of oxidative induced health complications.

Keywords: Anti-oxidant; flavanoid; phenol; poly-herbal extract

Abbreviations: ROS: Reactive Oxygen Species, **DPPH:** 1,1-diphenyl-2- picrylhydrazyl, **ANOVA:** One-way analysis of Variance.

I. INTRODUCTION

Organisms were exposed by exogenous oxidizing agents from environmental pollutants throughout their life span and to endogenous ones produced by metabolism. Chemical entities that behave as oxidizing agents possess reactive oxygen species (ROS), namely superoxide anion (O_2•-), hydroxyl (HO•), and peroxyl (ROO•) radicals, or reactive nitrogen species (RNS), which include agents like peroxynitrite anion (ONOO-) and nitric oxide (NO•) radical. In addition, non-free radical species also behave like oxidizing agents such as hydrogen peroxide (H_2O_2), nitric oxide (NO) and hypochlorous acid (HClO) [1].

Imbalance between oxidant and anti-oxidative system leads to oxidative stress. It is the risk factor for the development of various chronic diseases. Generation of free radicals and other reactive oxygen species are potent agents involved in the pathogenesis of numerous diseases like asthma, inflammatory, cancers as well as atherosclerosis. Aging in the human being is directly linked with reactive oxygen species [2].

Aromatic plants and its product have numerous medicinal qualities and possess valuable chemical constituents which have been used for medicinal purposes in all over the world. The phyto-constituent present in the plants acts synergistically for enhancement of immunity system and improving the memory, to cure stress and fatigue. Important plant parts like leaves, flower, bark, seed as well as roots are used naturally to cure disease. About 80% population of the world depends on the herbal medicine as the primary source for health care [3].

Generally poly-herbal formulations are used to make effective ayurvedic drug from ancient times because it provides synergistic therapeutic effects. Mentat is a fixed poly-herbal combination containing approximately 20 other different indigenous ingredients, widely used for treatment of many chronic disorders. The principle herbs present in the mentat are Brahmi (*Bacopa monnieri*), Shatavari (*Asparagus racemosus*), Shankhapushpi (*Evolvulus alsinoides*), Ashvagandha *(Withania somnifera),* Vacha *(Acorus calamus),* Amla (*Emblica officinalis),* and Triphala [4]. It is reported that mentat is an ayurvedic formulation which improve the memory and reduce the harmful effect of reactive oxygen species [5].

Poly-herbal preparations were used in treatment of health problems is based on the concept that ingredients may work in synergism. Compounds like flavonoids and phenolics that exists widely in many aromatic and medicinal plants and has enormous pharmacological potential to contribute an important role in the prevention of oxidative stress [6].

In the present study poly-herbal formulation was prepared using fresh leaves of *Lantana camara, Magnolia obovata, Albizzia lebbeck,* stem bark of *Clitoria ternatia, Cinnamomum cassia* and dry fruits of *Coriander sativum*. Anti-oxidant activity of poly herbal extract was evaluated by using four different assays such as total anti-oxidant activity, DPPH free radical scavenging activity, reducing power and hydrogen peroxide scavenging activity. We also estimated the

total polyphenol content in the poly-herbal formulation. Till date no any report exists on poly-herbal formulation using above plant parts.

II. MATERIAL AND METHODS

A. Sample collection and preparation of polyherbal extract

The fresh leaves of *Magnolia obovata, Albizzia lebbeck, Lantana camara* was collected from the campus of Banaras Hindu University, Varanasi in the month of August and September. The stem bark of *Cinnamomum cassia*, *Clitoria ternatia,* dry fruits of *Coriander sativum* was purchased from local market of Varanasi. The authentication was done by Dr. Jasmeet Singh, Department of Dravyaguna, Faculty of Ayurveda, Banaras Hindu University, Varanasi.

The collected plant materials (fresh leaves) of *Lantana camara, Magnolia obovata, Albizzia lebbeck,* was dried under shade and after complete drying the leaves of these plants, dried stem bark of *Cinnamomum cassia*, *Clitoria ternatia* and dried fruits of *Coriandrum sativum*, were powdered in a mixer grinder until a suitable sizes is achieved. Then it was made to pass through sieve number 22/60. The powdered sample was used for the extraction. Assembled the neat and clean the soxhlet apparatus. The mixture of all these herbs weighed in equal ratio (50g/herb, 300g) and then it was defatted with petroleum ether. Later it was extracted with 600 ml Hydro-alcoholic solvent (50:50). The temperature of the heating mental was maintained at 45°C and extraction was continued for nearly about 6 hrs for 1 day only. After that the filtrate was evaporated to dryness at 40°C. Then it was kept in desiccators for removal of moisture for one week. The extract was stored for further phyto-chemical analysis and pharmacological studies.

B. Estimation of total phenol content (TPC)

The estimation of TPC was carried out by the Folin-Coicalteau calorimetric methods [7]. The 100 mg of poly-herbal extract was dissolved in 100ml of triple distilled water (TDW) and filtered. To 1ml of polyherbal solution, 0.5ml of Folin-Coicalteau reagent (1:1 dilution) and 1.5ml of sodium carbonate was added in a test tube and volume was made up to 10 ml with TDW. Blank solution was also prepared in the same way excluding the sample. Absorbance was recorded against blank at 765nm using UV spectrophotometer. The total phenol content (TPC) was expressed in terms of mg/gm gallic acid equivalent.

C. Estimation of total tannin content (TTC)

Total tannin content estimation was done according to the method of Muniyandi *et al.* (2019) [8]. Accurately weighed 100mg of poly-herbal extract was taken and dissolved in triple distilled water (TDW) in a 200ml volumetric flask and the volume was made up to 100ml. To 1ml of this solution, 8ml of TDW, 0.5ml of FC reagent (1:1 dilution) and 1.5ml of sodium carbonate was added in a test tube. Absorbance was recorded against blank at 725 nm using UV spectrophotometer. The total tannin content was expressed in terms of mg/gm tannic acid equivalent (TAE).

D. Estimation of total flavonoid content (TFC)

Determination of total flavonoid content was done using aluminium chloride method. Rutin used as the standard reference compound [8]. The method is based on the formation of a flavonoid-aluminium complex having the absorption maxima at 415nm. From the plant extract solution of 10mg/ml concentration, 100 µl was taken and mixed with 100µl of 20% aluminium chloride ($AlCl_3$) solution in methanol and a drop of acetic acid. This mixture is then diluted with methanol to make the final volume up to 5ml. After 40 minutes, absorbance was recorded at 415 nm against blank. All measurements were performed in triplicates.

E. Estimation of total flavonol content

The flavonol estimation was done according to the method proposed by [9]. From the hydro-alcoholic extract (10mg/ml) 1ml was taken and mixed with 1ml of aluminium chloride (20 mg/ml w/v $AlCl_3$) and 3ml of sodium acetate (50 mg/ml w/v). After 2.5 hours, absorbance was recorded at 440 nm against blank. The absorption of standard rutin solution (0.5 mg/ml w/v) in methanol was measured under the same condition against blank.

F. Determination of total anti-oxidant activity (TAO)

The anti-oxidant activity of the extracts was evaluated by the phospho-molybdenum method according to the procedure of [10]. Extract (0.3 ml) was mixed with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95.1ºC for 90 minutes. Cool the reaction mixture at room temperature. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer (Shimadzu, UV-150-02) against blank. Methanol (0.3 ml) in the place of extract is used as the blank.

G. DPPH free radical scavenging activity

The free radical scavenging activity of the extract, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by [11]. Poly herbal extract (0.1 ml) was added to 3ml of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 minutes, and the percentage inhibition activity was calculated from $[(A_0–A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample extract.

H. Hydrogen peroxide scavenging activity

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of [12]. A solution of hydrogen peroxide (2mmol/l) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectro-photometrically at 230 nm with molar absorptivity 81mol/l cm^{-1}. Extract (25-400 µg/ml) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage inhibition activity was calculated from equation; $[(A_0 –A_1)/A_0] \times 100$, where A_0 is the absorbance of the control andA_1 is the absorbance of the extract.

I. Reducing power assay

The reducing power of poly herbal extract was determined according to the method of [13]. Extract (100 µg) in 1ml of methanol was mixed with phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6) and potassium ferricyanide [K_3 $Fe(CN)_6$] (2.5 ml, 1%). The reaction mixture was incubated at 50ºC for 20 minuts. Trichloro acetic acid (TCA) (2.5 ml, 10%) was added to the

mixture, which was then centrifuged at 6000 rpm for 10 minutes at room temperature. The upper layer of solution (2.5 ml) was mixed with 2.5 ml distilled water and $FeCl_3$ (0.5 ml, 0.1%). Absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as positive control.

J. Statistical analysis

All data were recorded in triplicates. Statistical analysis of data was done by One-way analysis of variance (ANOVA) by SPSS 16. Data were expressed as Mean ± SEM. A level of *p< 0.05 was accepted as statistically significant.

III. RESULTS

In the present communication six medicinal plants were used for the preparation of poly-herbal formulation. Equal proportions of medicinal plant ingredients were mixed homogenously and hydro-alcoholic extract (50:50) were prepared by using soxhlet apparatus. After removal of moisture the % yield (w/w) was calculated and it was found to be 5.2% (15.6 gm). The anti-oxidant activity in the poly-herbal hydro-alcoholic extract was measured using different assays like total anti-oxidant power, DPPH free radical scavenging activity, hydrogen peroxide scavenging ability and reducing power activity

A. Estimation of total phenolic, tannin, flavonoid and flavonol content

The amount of phytochemical constituents like total phenol and tannin content were determined by linear regression methods The gallic acid equivalent was used as the reference to prepare standard curve ($y = 0.029x+0.118$, $R^2=0.998$) for the estimation of phenol. It was found that amount of phenolic content in poly herbal extract was 2.37 mg/gm gallic acid equivalent (Table 1). For the estimation of tannin content in the extract, tannic acid equivalent was used as a reference to prepare standard curve ($y = 0.029x+0.0118$, $R^2 =0.118$). The tannin content was found to be 0.96mg/gm tannic acid equivalent (Table 1). The amount of flavonoid as well as flavonol content in the poly herbal extract were expressed as rutin equivalent and was calculated by the formula $X= (A\times M_0 /A_0\times M)$; where X is the flavonoid/ flavonol content which is expressed as mg/gm equivalent to rutin, A is the absorbance of plant extract solution, A_0 is the absorbance of standard rutin solution, M is the weight of plant extract in mg and M_0 is the weight of rutin in the solution in mg. From the above formula it was found that the amount of total flavonoid and flavonol content in the poly herbal extract was 8.20 mg/gm equivalent to rutin and 6.98 mg/gm quercetrin equivalent respectively (Table 1).

Table 1: Quantitative estimation of phytoconstituents (total phenol, total tannin, total flavonoid and total flavonol) present in poly-herbal extract.

Phytoconstituents	Total quantity
Total phenol content	2.37 mg/gm gallic acid equivalent
Total tannin content	0.96 mg/gm tannic acid equivalent
Total flavonoid content	8.20 mg/gm rutin equivalent
Total flavonol content	6.98 mg/gm quercetrin equivalent

B. Determination of total anti-oxidant activity (TAO)

The phosphor-molybdenum method was based on the reduction of Mo (VI) to Mo (V) by the anti-oxidant compounds and the formation of a green phosphate-molybdenum-vanadium complex with a maximal absorption at 695 nm. Results revealed that ascorbic acid which was used as a standard showed mean absorbance value 0.241, the mean absorbance of poly herbal drug mentat and poly herbal extract was 0.232 and 0.217 respectively (Table 2).

C. DPPH free radical scavenging activity

The reduction capability of DPPH radical was determined by the decrease in absorbance at 517 nm induced by antioxidants.

Ascorbic acid was used as standard for comparing the results. The poly-herbal extract showed significant DPPH free radical scavenging activity. The EC_{50} value of poly-herbal extract was 104.74, while the ascorbic acid showed low EC_{50} value (45.99) followed by standard herbal drug mentat (77.17) (Table 3).

D. Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging ability of poly-herbal extract, rutin and mentat were measured. From the result it was observed that the poly-herbal extract has the capacity for significant hydrogen peroxide scavenging activity in a concentration-dependent manner.

Table 2: Total anti-oxidant activity of hydro-alcoholic extract of poly-herbal formulation.

Concentration (µg/ml)	Absorbance at 695 nm (Mean±SEM)		
	Ascorbic acid	Mentat (polyherbal standard drug)	Poly-herbal extract
25	0.120 ± 0.004*	0.116 ± 0.004*	0.106 ± 0.004*
50	0.189 ± 0.002*	0.180 ± 0.003*	0.164 ± 0.003*
75	0.201 ± 0.002*	0.189 ± 0.003*	0.180 ± 0.002*
100	0.272 ± 0.003*	0.260 ± 0.003*	0.223 ± 0.002*
125	0.424 ± 0.003*	0.419 ± 0.004*	0.412 ± 0.004*
Mean	**0.241**	**0.232**	**0.217**

*Significant at (P<0.01)

Table 3: DPPH free radical scavenging activity of hydro-alcoholic extract of poly-herbal formulation.

Concentration (µg/ml)	Percent inhibition (Mean±SEM)		
	Ascorbic acid	Mentat	Poly-herbal extract
25	26.92 ± 2.25**	22.27 ± 4.09ns	11.88 ± 2.25ns
50	62.62 ± 3.06**	34.57 ± 1.28**	19.44 ± 3.33*
75	84.50 ± 3.75**	49.78 ± 4.35**	29.52 ± 3.63**
100	94.85 ± 2.30**	71.53 ± 2.64**	53.31 ± 3.42**
200	102.65 ± 3.76**	102.68 ± 4.57**	97.69 ± 3.09**
EC_{50}	**45.99**	**77.17**	**104.74**

Non-significant (ns) (P>0.05);* Significant at (P<0.05); **Highly-significant at (P<0.01)

Table 4: Hydrogen peroxide scavenging activity of hydro-alcoholic extract of poly-herbal formulation.

Concentration (µg/ml)	Percent inhibition (Mean±SEM)		
	Rutin	Mentat	Poly-herbal extract
25	38.32 ± 2.58ns	18.32± 3.02*	37.11± 5.41ns
50	46.59 ± 3.05**	38.24± 3.42**	54.10± 3.11**
100	72.25 ± 1.70**	60.82± 4.89**	63.75 ± 3.08**
200	82.1 ± 3.25**	70.59± 1.94**	85.35 ± 3.96**
400	88.41 ± 2.76**	90.11± 4.62**	89.24 ± 2.55**
EC_{50}	**49.16**	**77.50**	**46.89**

Non-significant (ns) (P>0.05);* Significant at (P<0.05); **Highly-significant at (P<0.01)

The EC_{50} value of poly-herbal extract was 46.89, while rutin and mentat showed 49.16 and 77.50 respectively (Table 4). It is clear from the result that poly-herbal extract showed more effective hydrogen peroxide scavenging activity than rutin and mentat.

E. Reducing power (RP) activity

It is clear from the result that poly-herbal extract showed significant reducing power activity. Maximum reducing power activity was in this order: ascorbic acid (OD at 700nm 0.581±0.001) >mentat (OD at 700nm 0.546±0.004)>poly herbal (OD at 700nm 0.454±0.014) at 100µg/ml concentration (Fig. 1).

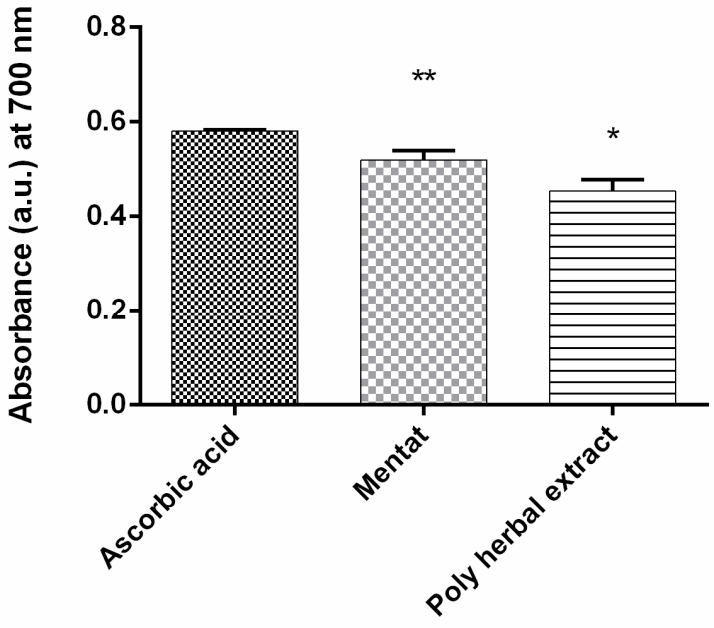

Fig. 1. Reducing power of poly herbal extract;* Significant at (P<0.05); **Highly-significant at (P<0.01).

IV. DISCUSSION

Medicinal plants are the major source of medicines due to the presence of various secondary metabolites. These plants possess many therapeutic activities. At present interest of the people towards herbal medicines were increased tremendously. Free radicals are highly unstable molecules having unpaired electron which are highly reactive with other molecules [14]. The elements namely oxygen and nitrogen participates in creating reactive oxygen species and reactive nitrogen species [15]. The reactive oxygen species are the free radicals

like hydroxyl radical ($OH^{\cdot}$), superoxide anion ($O_2^{\cdot-}$), hydro-peroxyl radical ($HO_2^{\cdot}$), singlet oxygen ($^1O_2$) [16]. In the various metabolic pathways the hydro peroxyl radicals dissociates and forms super oxide anion($O_2^{\cdot-}$) which can react with other molecule to generate reactive oxygen species(ROS) in the cellular environment [17].

Phenol, flavanoid, tannin and flavonol are natural anti-oxidant found in the plant and are capable to prevent the oxidation of other molecules [7]. For evaluation of phenol content Folin-Coicalteau (FC) assay was used in this study. It was observed that poly-herbal extract is rich with phenol content. Herbal extracts with high phenol content was reported in other species also [18-19]. Similarly, tannin content in the extract was also measured and it was revealed from the result that poly-herbal extract has significance tannin content. Flavanoid content in the poly-herbal extract was determined by comparison with standard drug rutin and it was observed that it is present at significant level in the poly-herbal formulation. Flavonoid is well known free radical scavengers due to the availability of phenolic hydrogen. Our result showed that the total extractable flovonol content was present at significant level. Flavonols, possess various pharmacological activities that contribute to health benefits and avert the oxidative stress in the cellular environment. Polyphenolic compounds have aromatic ring bearing hydroxyl group acts as anti-oxidants and showed free radical scavenging activity by chelation of metal ions and play prominent role in protective effects against chronic diseases [20-21]. The anti-oxidant properties in the poly-herbal extract is due to the presence of polyphenolic content.

Any chemical species having unpaired electron is known as free radical. Due to presence of unpaired electron these species are paramagnetic in nature and are highly reactive. Chemical reactivity of the free radicals is usually high in the cellular environment. Plants extracts has high free-radical scavenging capacity with wound healing and anti-aging properties. Phospho-molybdenum methods is a simple method used to determine the total antioxidant power in plant extract [22]. It is based on the reduction of Mo (VI) to Mo (V) by the poly-herbal formulation and form green color phospho-molybdenum-vanadium complex [9]. DPPH assay is the most common assay and used to determine the radical scavenging capacity. It showed a strong absorption at 517 nm due to its odd electron and solution appears a deep violet colour. The discoloration of reaction mixture is depends on the number of electrons taken up by poly-herbal extract. It is the simple method and widely applied to evaluate the ability of compound that act as free radical scavengers [23-24]. Hydrogen peroxide inactivates enzymes usually by oxidation of sulfhydryl (SH) groups [25]. It has capacity to cross cell membrane and react with Fe^{++} as well as Cu^{++} ions and generate hydroxyl radicals inside the cell. It showed toxic effects on biological molecules which are present in the cellular environment [26]. Reducing power activity is based on the principal that the extract which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) and then after reaction with ferric chloride form ferric-ferrous complex and showed maximum absorbance at 700nm [27]. High absorbance indicates the good reducing power in the poly-herbal formulation. Another study found general evidence for high absorbance indicates high reducing power [28]. The poly herbal formulation showed good anti-oxidant activity which acts synergistically as a good electron donor and prevent the formation of hydroxyl radical [29]. The combination of two or more substances showed higher anti-oxidant effects than the sum of the single substance. Good anti-oxidant activity of the poly-herbal formulation may be due to synergistic effect [30] of phyto-constituents. In the present study we found rich poly phenol content and good anti-oxidant activity of the formulation. In Indian system of medicine several formulations [31-32] were used for the treatment of several health complications. This indigenous novel formulation can also be use for bio-prospection and drug development.

V. CONCLUSIONS

The results of the present study showed that poly-herbal extract exhibits significant anti-oxidant activity. The data suggests that poly-herbal extract was rich with the phenol, flavonoid, tannin and flavonol content. Hydro-alcoholic extract of the poly herbal formulation possesses good anti-oxidant and free radical scavenging activity. So this formulation can be used in medication to reduce the side effects produced by the free radicals in the body of human being. Before its application in medication further its toxicity studies and clinical confirmation is required.

ACKNOWLEDGEMENT

Author is highly thankful to Head, Department of Botany, MMV, BHU, Varanasi and Dr. S.K. Mishra, Department of Pharmaceutical Engineering and Technology, IIT, BHU, Varanasi for availing necessary research facilities.

Conflict of interest: None

REFERENCE

[1]. Mosquera, O.M., Correra, Y.M. and Niño, J., (2009). Antioxidant activity of plant extracts from Colombian flora. *Brazilian Journal of Pharmacognosy*, **19**: 382-387.

[2]. Mahdi-Pour, B., Jothy, S.L., Latha, L.Y., Chen, Y. and Sasidharan, S., (2012). Antioxidant activity of methanol extracts of different parts of *Lantana camara*. *Asian Pacific Journal of Tropical Biomedicine*, **2**: 960-965.

[3]. Ekor, M., (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, **4**: 1-10.

[4]. Kumar, A. and Kulkarni, S.K. (2006). Effect of BR-16A (Mentat), a polyherbal formulation on drug-induced catalepsy in mice. *Indian Journal of Experimental Biology*, **44**: 45-48.

[5]. Viswanatha, G.L., Kumar, L.M.S., Rafiq, M., Kavya, K.J., Thippeswamy, A.H., Yuvaraj, H.C., Azeemuddin, M., Anturlikar, S.D., Patki, P.S., Babu, U.V. and Ramakrishnan, S., (2015). LC MS/MS profiling and neuroprotective effect of Mentat® against transient global ischemia and reperfusion-induced brain injury in rats. *Nutrition*, 1-41. doi: 10.1016/j.nut.2015.02.009.

[6]. Tungmunnithum, D., Thongboonyou, A., Pholboon, A. and Yangsabai, A., (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. *Medicines*, **5**: 1-16.

[7]. Singleton, V.L., Orthofer, R. and Lamuela, R.R.M., (1974). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods of Enzymology*, **229**: 152-178.

[8]. Muniyandi, K., George, E., Sathyanarayanan, S., George, B.P., Abrahamse, H., Thamburaj, S. and

Thangaraj, P. (2019). Phenolics, tannins, flavonoids and anthocyanins contents influenced antioxidant and anticancer activities of *Rubus* fruits from Western Ghats, India. *Food Science and Human Wellness*, **8**: 73-81.
[9]. Kumaran, A. and Karunakaran, R.J., (2006). Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chemistry*, **97**: 109-114.
[10]. Prieto, P., Pineda, M. and Anguilar, M., (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, **269**: 337-341.
[11]. Braca, A., Tommasi, N.D., Bari, L.D., Pizza, C., Politi, M. and Morelli. I. (2001). Antioxidant principles from *Bauhinia terapotensis*. *Journal of Natural Products*, **64**: 892-895.
[12]. Ruch, R.J., Cheng, S.J. and Klaunig, J.E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, **10**: 1003-8.
[13]. Oyaizu, M., (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japan Journal of Nutrition*, **44**: 307-315.
[14]. Kumar, R. and Sharma, S., (2018). *In-vitro* Free radical scavenging potential of flavonoids with synergistic effect of their combination. *Journal of Drug Delivery and Therapeutics*, **8**: 200-203.
[15]. Phaniendra, A., Jestadi, D.B. and Periyasamy, L., (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, **30**: 11–26.
[16]. Gupta, M., Karmakar, N. and Sasmal, S., (2017). *In vitro* antioxidant activity of aqueous and alcoholic extracts of polyherbal formulation consisting of *Ficus glomerata* Roxb. And *Symplocos racemosa* Roxb. stem bark assessed in free radical scavenging assays. *International Journal of Pharmacognosy and Phytochemical Research*, **9**: 181-189.
[17]. Kasote, D.K., Katyare, S.S., Hegde, M.V. and Bae, H., (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences*, **11**: 982-991.
[18]. Chanthasri, W., Puangkeaw, N., Kunworarath, N., Jaisamut, P., Limsuwan, S., Maneenoon, K., Choochana, P. and Chusri, S. (2018). Antioxidant capacities and total phenolic contents of 20 polyherbal remedies used as tonics by folk healers in Phatthalung and Songkhla provinces, Thailand. *BMC Complementary and Alternative Medicine*, **18**: 1-11.
[19]. Ely, Zayova, Maria, Petrova, Milena, Nikolova and Lyudmila, Dimitrova., (2016). Effect of Medium Salt Strength on the Micropropagation, Phenolic Content and Antioxidant Activity of *Arnica montana* L., Threatened Plant Species. *Bio Bulletin*, **2**(1): 6-13.
[20]. Alireza, Pazoki (2015). Evaluation of Flavonoids and Phenols content of Wheat under different Lead, PGPR and Mycorrhiza levels. *Biological Forum – An International Journal*, **7**(1): 309-315.
[21]. Mishra, A.K., Upadhyay, R., Chaurasia, J.K. and Tiwari, K.N., (2016). Comparative anti-oxidant study in different flower extracts of *Nyctanthes arbor-tristis* L. (Oleaceae): an important medicinal plant. *Brazilian Journal of Botany*, **39**: 813-820.
[22]. Naseem, Z., Alim-un-Nisa., Imran, Kalim, Muhammad, Khalid, Saeed, Ijaz, Ahmad and Sajila, Hina, (2017). Nutritional Evaluation and Antioxidant Activity of Zest obtained from Orange (*Citrus sinensis*) Peels. *International Journal of Theoretical & Applied Sciences*, **9**(1): 7-10.
[23]. Llorent Martinez, E., Ortega Barrales, P., Zengin, G., Mocan, A., Simirgiotis, M., Ceylan, R., Uysal, S. and Aktumsek, A., (2017). Evaluation of antioxidant potential, enzyme inhibition activity and phenolic profileof *Lathyrus cicero* and *Lathyrus digitatus*: potential sources of bioactive compounds for the food industry. *Food and Chemical Toxicology*, **107**: 609–619.
[24]. Upadhyay, R., Chaurasia, J.K., Tiwari, K.N., and Singh, K., (2014). Antioxidant property of aerial parts and root of *Phyllanthus fraternus* Webster, an important medicinal plant. *The Scientific World Journal*, 1-7. doi.org/10.1155/2014/692392.
[25]. Bindoli, A., Fukuto J.M. and Forman, H.J. (2008). Thiol chemistry in peroxidase catalysis and redox signaling. *Antioxidants & Redox Signaling*, **10**: 1549–1564.
[26]. Nagonda, F., (2013). *In- vitro* anti-oxidant activity and free radical scavenging potential of roots of Malawian *Trichodesma zeylanicumm* (burm.f.). *Asian Journal of Biomedical and Pharmaceutical Sciences*, **3**: 21-25.
[27]. Sharma, P.R. and Laware, S.L., (2016). Antioxidant and anti-lipid peroxidation potential of polyherbal formulation. *Journal Pharmaceutical Science and Research*, **8**: 47-52.
[28]. Upadhyay, R., Chaurasia, J.K., Tiwari, K.N. and Singh, K., (2013). Comparative antioxidant study of stem and stem induced callus of *Phyllanthus fraternus* Webster- an important antiviral and hepatoprotective plant. *Applied Biochemistry and Biotechnology*, **171**: 2153-2164.
[29]. Wang, S., Meckling, K.A., Marcone, M.F., Kakuda, Y. and Tsao, R., (2011). Synergistic, additive, and antagonistic effects of food mixtures on total antioxidant capacities. *Journal of agricultural and food chemistry*, **59**: 960-968.
[30]. Rahim, N.F.A., Muhammad, N., Putra, T.N.M.T., Rashidi, W.N.A.S.W.M., Talip, B.H.A., Abdullah, N. and Bakar, M.F.A., (2018). Interaction effect of polyherbal formulations with antioxidant activity. *Eurasian Journal of Analytical Chemistry*, **13**: 613-618.
[31]. Petchi, R.R., Vijaya, C. and Parasuraman, S. (2014). Antidiabetic activity of polyherbal formulation in streptozotocin – nicotinamide induced diabetic Wistar rats. *Journal of Traditional and Complementary Medicine*, **4**: 108-117.
[32]. Devaraj, V.C., and Krishna, B.G., (2013). Antiulcer activity of a polyherbal formulation (PHF) from Indian medicinal plants. *Chinese journal of natural medicine*, **11**: 145-148.

How to cite this article: Saini, R., Mishra, A.K., Mishra, S.K. and Tiwari, K.N. (2019). Evaluation of Polyphenolic Content and Anti-oxidant Potential of Poly-herbal Formulation. *International Journal of Emerging Technologies*, **10**(2): 243–248.