International Journal of Pharmacognosy and Pharmaceutical Sciences
Online ISSN: 2706-7017; Print ISSN: 2706-7009
Received: 01-11-2018; Accepted: 03-12-2018; Published: 03-01-2019
www.pharmacognosyjournal.net
Volume 1; Issue 1; 2019; Page No. 01-04

Phytochemical Screening and *In-vitro* Antioxidant Potential of a Polyherbal Formulation

Prakash Mishra[1], Anas Khan[2], Ashra Khokar[3], Sarita Karole[4*], Sarika Shrivastava[5], Anup Chakraborty[6]
[1-6] Oriental College of pharmacy, Raisen Rd, Patel Nagar, Bhopal, Madhya Pradesh, India

DOI: https://doi.org/10.33545/27067009.2019.v1.i1a.7

Abstract
Plant extracts contains huge number of chemical compounds, making them a rich source of different types of medicines. Thus, a proper scientific evidence or assessment has become the criteria for acceptance of herbal health claims. As such developing a polyherbal formulation will definitely produce synergistic effect as needed comparable to single extract or standard drugs that are available in market all over the world. Plants are the richest source for antioxidant and are effective in the management of oxidative stress, caused by free radical damage. The objective of this study is to determine phytochemical and the optimum antioxidant properties of polyherbal formulations of the methanolic extract of leaves of *Phyllanthus emblica* (Amla), *Moringa oleifera* (Drumstick) and *Citrus limon* (Lemon) using *in vitro* method. Phytochemical screening showed the presence of alkaloids, glycosides, carbohydrates, amino acid, tannin, steroids, and flavonoids in the individual extracts. The antioxidant study of the individual extracts and polyherbal formulation were conducted using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay method. Synergy is the interaction of two or more extracts to produce a combined effect greater than the totality of their individual effects. The results suggest that *P. emblica* has higher potential of antioxidant properties. Synergistic effect was exhibited in polyherbal formulation. In conclusion, the results indicated that the formulations obtained in this present study justify the promising antioxidant properties of the polyherbal mixtures. Thus, it is sensible to use the combination of studied herbal formulation for development of any antioxidant supplement or food products in the future.

Keywords: polyherbal formulations, *Phyllanthus emblica*, *moringa oleifera*, *citrus limon*, DPPH

1. Introduction
High margin of safety, cost effective, eco-friendly and readily availability, had caused increasing development of herbal supplement involving traditional medicinal plants [1]. The medicinal plants are expected to have benefits such as a radical scavenging activity inhibitor, known as antioxidant. In nutraceutical and pharmaceutical industries, antioxidant plays a significant function as a health protecting factor which may reduce the risk of oxidative stress-related diseases and able to give health-enhancing effect on human [2]. The main characteristic of an antioxidant is its ability to trap free radicals which referred to the oxygen-centered molecules that contain a single electron at the outermost orbit. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources [3]. Thus, the study of biological activity and chemical composition of medicinal plant extracts as a potential source of natural antioxidants are becoming a trend in development of product. Lemon is among the most important crops in the world, with an annual production of about 123 million tons in 2010. Lemon (*Citrus limon* L.) occupies the third most important *Citrus* species after orange and mandarin world production by 4.200.000 metric tons [4]. Lemon (Rutaceae) has many important natural chemical components, including citric acid, ascorbic acid, minerals, and flavonoids. Although its health-related properties have always been associated with its vitamin C content, it has recently been shown that flavonoids also play a role in this respect. Some authors suggest that flavonoids have different biological functions, including antioxidative, anti-inflammatory, antiallergic, antiviral, antiproliferative, antimutagenic, and anticarcinogenic activities [5, 6]. Therefore, although the new *Citrus* cultivars have mainly been selected and developed for fresh consumption, the particular characteristics of their flavonoid contents have led to their use in pharmacological and food technology areas [7, 8]. Lemon fruit contains very important natural chemical components, including phenolic compounds (flavonoids) and other nutrients and non-nutrients (vitamins, minerals, dietary fiber, essential oils (EO), and carotenoids). The health-promoting effects and properties have been associated with the contents of vitamin C and flavonoids, due to their natural antioxidant characteristics. Overall, lemon fruits, rich in flavonoids, are a very important part of a balanced diet, particularly for their role in prevention of diseases, such as obesity, diabetes, blood lipid lowering, cardiovascular diseases, and certain types of cancer [9]. *Moringa oleifera* belongs to the family Moringaceae which contains around 13 species [10 11]. It is native to Africa, Asia Minor, the Indian subcontinent [12] and is distributed in the Philippines, Cambodia, Central America, North and South America, and the Caribbean Islands [13]. The most commonly known species is the *M. oleifera* Lam., which is also known as drumstick tree [14]. Different parts of *M. oleifera* are used for various purposes such as, Fertilizer, green manure, animal feed, medicine, foliar nutrient, bio-pesticide, water purifier; sources of vitamin C are different uses of this plant [15]. Moringa seed is used for decontamination and treatment of high turbid water [10]. *M. oleifera* seeds contain 33–41% w/w vegetable oil [16] and this oil is very much similar to the olive oil and contain every single unsaturated fat contained in olive oil, aside from linoliec. It is medicinally useful [17]. Moringa seed oil, has a defensive activity against poisonous impacts [18]. It is broadly used as a part of cooking, lighting, hairdressing, cleanser and scent in commercial enterprises. The *M. oleifera* seed shown antimicrobial, anti-

inflammatory, anti-cancer, hepatoprotective, diuretic activity, antiasthmatic and anti-diabetic activity [15]. The fruits of *Phyllanthus emblica* (Euphorbiaceae), commonly known in India as amla, are consumed as fruit or in the form of food products. Traditionally, the fruit is useful as an astringent, cardiac tonic, diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, alterative, antipyretic, anti-inflammatory, hair tonic, and digestive medicine [19]. It is reported to have hepatoprotective activity against carbon tetrachloride [20], anti-tumor [21], radioprotective effect against gamma irradiation [22], chemopreventive potential for hepatocarcinogenesis [23], antiproliferative [24] and gastroprotective [25] activities. Polyphenols are widely distributed in plants and phenolic antioxidants have been found to act as free radical scavengers as well as metal chelators [26]. Among the diverse roles of polyphenols, they protect cell constituents against destructive oxidative damage, thus limiting the risk of various degenerative diseases associated with oxidative stress and thus tending to be potent free radical scavengers. Their ability to act as antioxidants is due to their chemical structure and ability to donate/accept electrons, thus delocalizing the unpaired electron within the aromatic structure [27]. Basically, plant extracts are natural component that might employ synergistic, antagonistic, additive and indifferent effect depending on the interaction on the phytochemicals [28]. In addition, combining of the plant extracts can also produce all those effects. To date, scientists are still exploring the possibilities of combining effect of the plant [29]. Synergistic effect is defined as a positive interaction when a combination of two or more substances shows higher mechanism than the sum of the single substances [30]. In other word, it is a new concept in development of food product from natural sources which can give optimum antioxidant effect due to harmful effects of synthetic antioxidants on human health [31]. Therefore, this study aims to investigate the synergistic effect of leaves extract of *M. oleifera*, *P. emblica* and *C. limon* for polyherbal formulation followed by evaluation of their interaction effect towards antioxidant activity.

Materials and Methods

Plant material

Leaves of *M. oleifera*, *P. emblica* and *C. limon* were collected from local area of Bhopal (M.P.) in the month of March, 2019.

Chemical reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid were purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI, USA). All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study was of analytical grade.

Extraction

Defatting of plant material

Powdered plant material of *M. oleifera*, *P. emblica* and *C. limon* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

100 gm of *M. oleifera*, *P. emblica* and *C. limon* dried leaves were exhaustively extracted with methanol using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [32, 33]. 25 mg of each extract was mixed together and henceforth labeled as Polyherbal formulation. This mixture of 25mg each of residue was dissolved in 10ml of methanol and, boiled in water bath for 5 minutes, cooled and centrifuged at 4000 rpm for 10 minutes. The clear supernatant was used for evaluating antioxidant properties in various assays.

Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures [34, 35]. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

Antioxidant activity using DPPH method

DPPH scavenging activity was measured by modified method [36]. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

Results and Discussion

The crude extracts so obtained after the maceration extraction process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. The percentage yield of extract was given in Table 1. Phytochemical analysis of methanolic extracts of plants showed the presence of flavonoid, phenol, alkaloids, carbohydrate and saponins while, protein, glycosides and oils and fats were reported to be absent Table 2. Antioxidant activity of the samples was calculated through DPPH assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the %

inhibition the better the activity. Ascorbic acid was taken as standard and the values were comparable with concentration ranging from 50 µg/ml to 300µg/ml. A dose dependent activity with respect to concentration was observed Table 3. The results suggest that *P. emblica* has higher potential of antioxidant properties than other extract. Synergistic effect was exhibited in polyherbal formulation.

Table 1: % Yield of methanolic extract

S. No.	Extracts	% Yield (w/w)
1	*Moringa oleifera* extract	6.98
2	*Phyllanthus emblica* extract	5.77
3	*Citrus limon* extract	7.23

Table 2: Result of phytochemical screening of methanol extracts

S. No.	Constituents	*M. oleifera*	*P. emblica*	*C. limon*
1.	Alkaloids	-ve	+ve	+ve
2.	Glycosides	-ve	-ve	-ve
3.	Flavonoids	+ve	+ve	+ve
4.	Diterpenes	+ve	+ve	+ve
5.	Phenolics	+ve	+ve	+ve
6.	Amino Acids	-ve	+ve	-ve
7.	Carbohydrate	+ve	+ve	+ve
8.	Proteins	-ve	+ve	-ve
9.	Saponins	+ve	+ve	+ve
10.	Oils and fats	-ve	-ve	-ve

Table 5: % Inhibition of ascorbic acid, methanolic extract and polyherbal formulation using DPPH method

S. No.	Conc. (µg/ml)	Ascorbic acid % Inhibition	*P. emblica* % Inhibition	*C. limon* % Inhibition	*P. emblica* % Inhibition	Polyherbal F % Inhibition
1	50	21.68±0.82	19.31±0.55	22.49±1.36	18.24±1.47	23.30±0.58
2	100	33.7±1.90	24.29±0.49	24.19±1.15	22.72±1.76	32.25±1.19
3	150	44.76±0.97	32.41±0.59	30.44±1.43	26.72±1.53	43.33±0.64
4	200	54.96±2.65	43.31±0.722	43.32±0.63	38.36±1.21	56.45±2.14
5	250	69.72±1.43	51.77±0.90	53.49±0.53	49.3±1.48	63.87±1.23
6	300	81.93±0.91	66.75±2.09	62.75±1.16	62.43±1.22	72.8±1.12
	IC 50	162.97±3.39	230.15±3.64	236.04±4.53	251.98±5.88	181.66±2.77

Conclusion

It can be concluded that from present investigation. The phytochemical investigation gave valuable information about the different phytoconstituents present in the plant, which helps the future investigators concerning the selection of the particular extract for further investigation of isolating the active principle and also gave idea about different phytochemical have been found to possess a wide range of activities. It can be concluded polyherbal formulations of the methanolic extracts in this study showed the synergistic interaction towards DPPH radical scavenging activity. Thus, this finding may leads to the understandable product development in the future particularly in the studied herbs.

Conflicts of interest

The authors have none to declare.

References

1. Jain SK, Indian J. Trad Knowl. 2006; 2:321-332.
2. Adorjan B, Buchbauer G. Flav Frag J. 2010; 25(6):407-426.
3. Lobo V, Patil A, Phatak A, Chandra N. Pharmacog Rev. 2010; 4(8):118.
4. Gonzalez-MolinaE, Dom ´ınguez-Perles R, Moreno DA, Garcıa-Viguera CJ. Pharm Biomed Anal. 2010; 51(2):327-345.
5. Elangovan V, Sekar N, Govindasamy S. Cancer Lett. 1994; 87:107-113.
6. Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del Rio JA. J Agric Food Chem. 1997; 45:4505-4515.
7. Ortuno A, Reynaldo I, Fuster MD, Botia J, Puig DG, Sabater F, Lidon AG, Porras I, Del Río JA. Sci Hortic. 1997; 68:231-236.
8. Del Rio JA, Arcas MC, Benavente-Garcia O, Ortuno A. J Agric Food Chem. 1998; 46:4423-4428.
9. Campelo LML, Gonçalves FCM, Feitosa CM, de Freitas RM. Pharm Biol. 2011; 49:709-715.
10. Anwar F, Latif S, Ashraf M, Gilani AH. Phytother Res. 2007; 21:17-25.
11. Janick J, Paull RE. The Encyclopedia of Fruit and Nuts. CABI, Cambridge, 2008; 509-510.
12. Somali M, Bajneid M, Al-Fhaimani SJ. Am Oil Chem Soc. 1984; 61:85-86.
13. Morton J. Econ Bot. 1991; 45:318-333.
14. Awodele O, Oreagbaa IA, Odomaa S, Teixeira da Silva JA, Osunkaluc VO. J Ethnopharmacol. 2012; 139(2):330-336.
15. Jahan IA, Hossain MH, Ahmed KS, Sultana Z, Biswas PK, Nada K. Oriental Pharm Expl Med 18 https://doi.org/10.1007/s13596-018-0333-y
16. Rashid U, Anwar F, Moser BR, Knothe G. Bioresour Technol. 2008; 99:8175-8179.
17. Lalas S, Tsaknis JJ. Am Oil Chem Soc. 2002; 79(7):677-683.
18. Sanchez-Machado DI, Nunez-Gastélum JA, Reyes-Moreno C, Ramírez-Wong B, Lopez-Cervantes J. Food Anal Methods. 2010; 3:175-180.
19. Perianayagam J, Sharma S, Joseph A, Christina A. J. Ethnopharmacol. 2004; 95:83-85.
20. Jose JK, Kuttan R. J Clinical Biochem Nut. 1995; 19:63-70.
21. Jose JK, Kuttan G, Kuttan R. J Ethnopharmacol. 2001; 75:65-69.
22. Singh I, Soyal D, Goyal PK. J Env Pathol Toxicol Oncol. 2006; 25:643- 654.
23. Sultana S, Ahmed S, Jahangir T. J Ethnopharmacol. 2008; 118:1-6.
24. Khan MT. *et al*. Int J Oncol 2002; 21:187-192.
25. Al-Rehaily AJ, Al-Howiriny TA, Al-Sohaibani MO, Rafatullah S. Phytomedicine. 2002; 9:515-522.
26. Juang LJ, Sheu SJ, Lin TC. J Seperation Sci. 2004; 27:718-724.
27. Ghosh A, Sil PC. Biol Pharm Bull. 2008; 31:1651-1658.
28. Wang S, Meckling KA, Marcone MF, Kakuda Y, Tsao R. J Agric Food Chem. 2011; 59(3): 960-968.
29. Brigitte L, Emmanuel T, Kouam J, Zra T, Louis OEJ. Int J Biol. 2013; 5(3): 64-72.

30. Blesson J, Saji CV, Nivya RM, Kumar R. Aureus (Mrsa). 2015; 4(3):741-763.
31. Sheng ZW, Ma WH, Gao JH, Bi Y, Zhang WM, Duo HT, Jin ZQ. African J Biotech. 2011; 10(2):4470- 4477.
32. Mukherjee PK. Quality Control of Herbal Drugs, 2nd Edition, Business Horizons, 2007, 2-14.
33. Kokate CK. Ed. Practical Pharmacognosy, 4th Edn., Vallabh Prakashan. 1994; 112:120.
34. Khandelwal KR. Practical pharmacognosy technique and experiments. 23rd Ed. Nirali Prakashan, 2005.
35. Kokate CK. Practical pharmacognosy. 4th Ed. Vallabh Prakashan, 1994.
36. Olufunmiso OO, Afolayan AJ. BMC Comp Alter Med. 2011; 11:130.