JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH

RESEARCH ARTICLE

EVALUATION OF ANTIOXIDANT POWER OF POLYHERBAL FORMULATION

SHIVAKUMAR S. LADDE[1], BHUSNURE O. G[2*]

[1]*Reseach Scholar, Research Center of Channabasweshwar Pharmacy College, Latur- 413512, Maharashtra, India*

[2]*Dept. of Quality Assurance, Channabasweshwar Pharmacy College (Degree), Latur- 413512, Maharashtra, India, Email: shivkumarladde@gmail.com*

**Corresponding Author*

ABSTRACT

Oxidative stress is a pathological state that is responsible for the growth of chronic disorders like arteriosclerosis, cancer, diabetes mellitus, liver injury, inflammation, skin damages, coronary heart diseases, and arthritis. Antioxidants are substances that when found in food or herbs, that stop and stabilize the oxidative damage triggered by free radicals by providing electrons from antioxidants to these damaged cells. Ayurveda is said to be holistic as it objectives to integrate and balance body, mind, and spirit to stop illness and promote wellness, longevity, vitality, and happiness, hence in the current research we are planning to prepare and assess the efficacy of the total antioxidant potential and radical scavenging activity of a polyherbal formulation. The total antioxidant power and free radical scavenging activity of polyherbal formulation was assessed by Total Antioxidant Capacity by Phosphomolybdate Assay and DPPH Scavenging Activity. In Phosphomolybdate Assay the polyherbal formulation PHF2 shows the increasing total antioxidant capacity equivalence to Ascorbic acid per gram (µg AAE/g) with increasing concentration as compared to polyherbal formulation PHF1 and PHF3. While in DPPH free radical Scavenging assay, the polyherbal formulation (PHF2) shows highest free radical scavenging activity with IC50 = 43.57 µg/ml when evaluated with standard Ascorbic acid 48.25 µg/ml, PHF3 62.75 µg/ml and PHF1 88.15 µg/ml. Hence, the evolved polyherbal formulation might substantiate to be a safe alternative treatment for the management of chronic metabolic disorders like diabetes, cancer, liver injury, inflammation, skin damages, coronary heart diseases, and arthritis through reducing oxidative stress.

KEYWORDS:
Oxidative Stress, Chronic Disorder, Polyherbal Formulation, Ascorbic Acid, IC_{50}

ARTICLE HISTORY:
Received Aug 22, 2021
Accepted Sep 10, 2021
Published Oct 31, 2021

DOI:
10.5455/jcmr.2021.12.03.12

VOLUME: 12
ISSUE: 3
ISSN: 2146-8397

INTRODUCTION

Oxidative stress is a pathological state that is responsible for the growth of chronic disorders like arteriosclerosis, cancer, diabetes mellitus, liver injury, inflammation, skin damages, coronary heart diseases, and arthritis (Resat Apak, 2016) (Dontha, 2016). Oxidative stress is a condition where oxidants overwhelm the antioxidant protective system, which may lead to DNA damage and cellular lipid peroxidation (Garland K. More, 2020). Oxidative stress is owing to the discrepancy between the production of reactive oxygen species (ROS) alongside reactive nitrogen species (RNS) and the antioxidant defenses (Maria Agostina Frezzini, 2019).

Antioxidants are substances that when found in food or herbs or in the body at very low concentrations that stop and stabilize the oxidative damage triggered by free radicals by providing electrons from antioxidants to these damaged cells (Mahbubur Rahman, 2015). Antioxidants that fit in this definition encompass free radical scavengers, singlet oxygen quenchers, inactivators of peroxides and other reactive oxygen species (ROS), metal ion chelators, quenchers of secondary oxidation products, and inhibitors of pro-oxidative enzymes, among others (Fereidoon Shahidi, 2015). Plants are loaded in antioxidants; so considerable attention has been directed towards the growth of ethnomedicines as they contain phenols, flavonoids, alkaloids, tannins, vitamins, terpenoids, and numerous phytochemicals responsible for different pharmacological activities. Current research has proofed that ingestion of natural antioxidants has been linked with a reduced risk of cancer and many chronic diseases (Mayank Gangwar, 2014).

The philosophy behind Ayurveda is preventing needless suffering and living a long healthy life. Unlike the allopathic medicines which adopt mainly synthetic chemicals designed

for specific target receptors and mainly give symptomatic relief, Ayurveda involves the aid of natural means such as diet, herbs, spices, minerals, exercise, meditation, yoga, mental hygiene, sounds, smells, and mechano-procedures to eradicate the root origin of the disease by restoring balance, at the identical time generate a healthy lifestyle to stop the reoccurrence of imbalance. Ayurveda is said to be holistic as it objectives to integrate and balance body, mind, and spirit to stop illness and promote wellness, longevity, vitality, and happiness, hence in the current research we are planning to prepare and assess the efficacy of the total antioxidant potential and radical scavenging activity of a polyherbal formulation.

MATERIALS AND METHODS

Drugs, Chemicals, and Instruments

The gift sample of hydroalcoholic extract of *Allium Sativum, Punica Granatum, Zingiber Officinale*, and *Syzygium Cumini* provided by Kisalaya Herbals Limited, Indore, India, 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) extrapure, 95%, L-Ascorbic Acid extrapure AR, 99.7%, Sodium Phosphate Dibasic Anhydrous extrapure AR, 99%, Sodium Phosphate Monobasic Dihydrate extrapure AR, 99%, Ammonium Molybdate Tetrahydrate extrapure AR, 99%, were purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. Sulphuric Acid, Methanol, Distilled Water, Spectrophotometer (UV-1800 Shimadzu), RX-50V Semi Auto Biochemistry Analyzer, Micro Lab, Ahmedabad, India.

Development of Polyherbal Formulation

The polyherbal formulation was evolved by combining the dried hydroalcoholic extracts of the plant materials based on the oral glucose tolerance test of individual plant extracts (200 mg/kg each) in normal rats and total antioxidant capacity, advantageous in routine life (nutritional value) and reported activities of plants. The polyherbal formulation was made by mixing Hydroalcoholic Extract of *Allium Sativum* (bulb), *Punica Granatum* (Fruit), *Zingiber Officinale* (Shunt), and *Syzygium Cumini* (Seeds) in the proportion given in below table 01

Table 1: The hydroalcoholic extracts of *Allium Sativum* (A), *Punica Granatum* (B), *Zingiber Officinale* (C), and *Syzygium Cumini* (D) combinations

Prepared Combinations	
Name of Polyherbal Formulation	**Combination**
PHF1	Drug A: Drug B: Drug C: Drug D, 1:1:1:1
PHF2	Drug A: Drug B: Drug C: Drug D, 1.5:0.5:0.5:1.5
PHF3	Drug A: Drug B: Drug C: Drug D, 1:1:0.5:1.5

Estimation of Total Antioxidant Capacity (TAC) of Polyherbal Formulation by Phosphomolybdate Method (Mahbubur Rahman, 2015)

The total antioxidant capacity (TAC) of samples was determined by the technique reported by Prieto et al (1999) with little modification (Chutulo EC, 2020). The assay is based on the decrease in Mo (VI) to Mo (V) by samples and the formation of a green-colored phosphate/ Mo (V) complex at acidic pH.

Preparation of Molybdate Reagent Solution (Rohan Sharadanand Phatak, 2014)

1ml each of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was added in 20 ml of distilled water and made up the volume to 50 ml by adding distilled water.

Procedure

Hydroalcoholic extract of *Allium Sativum, Punica Granatum, Zingiber Officinale, Syzygium Cumini*, and polyherbal formulation (PHF1-3) in a different concentration varying from 50 to 250 μl were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95 °C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 765 nm by using Spectrophotometer (UV-1800 Shimadzu). Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as a positive reference standard.

Total Antioxidant Capacity (TAC) of the polyherbal formulation is expressed as Total Antioxidant Capacity Ascorbic Acid Equivalence per gram (AAE/g). A calibration curve of standard reference was pioneered using Ascorbic Acid (range of concentration from 50 to 250 μg/mL) as standard references plotted. TAC was elucidated as Ascorbic Acid equivalents in milligrams per gram of the polyherbal formulation (Renuka Diwan, 2012), (Rahmat Ali Khan, 2012), (Atina Rizkiya Choirunnisa, 2016), (Shumaila Jan, 2013).

Evaluation of DPPH (Diphenyl Dipicryl Hydrazide) Scavenging Activity of Polyherbal Formulation (Mahbubur Rahman, 2015), (Mayank Gangwar, 2014)

DPPH inhibition in MPE was determined by utilizing the protocol of Brand-Williams et al., with some modifications. The DPPH radical (SRL Chem) is stable owing to the delocalization of a spare electron over the molecule, consequently preventing dimer formation. This radical is utilized in the DPPH radical scavenging capacity assay to estimate the power of antioxidants to quench the DPPH radical. The dark purple color of DPPH will be lost when it is decreased to its nonradical form stable organic nitrogen centered free radical with a dark purple color which when reduced to its nonradical form by antioxidants becomes colorless. DPPH radicals are extensively utilized in the model system to investigate the scavenging activities of numerous natural compounds. When the DPPH radical is scavenged, the

color of the reaction mixture deviations from purple to yellow with decreasing absorbance at wavelength 515 nm (Resat Apak, 2016).

10- 250 µg/ml of sample was taken in the test tube and volume up to 3000 µl with Methanol. Three hundred microliter methanol was taken in blank instead of the sample. Then 1 mL of 0.004% DPPH (4 mg/100 mL of Methanol) solution was added to the sample and the blank. This setup was left at room temperature for 120 minutes and the absorbance was taken at 515 nm against the methanol by utilizing a UV-1800 spectrophotometer (Shimadzu, Japan). Each crude extract was analyzed in triplicate.

% DPPH radical scavenging activity = {(A0− A1)/A0} × 100

Where A0 is the absorbance of the control, and A1 is the absorbance of the extractives/standard. Then % of inhibition was plotted against concentration, and from the graph, IC50 was calculated. Ascorbic acid is adopted as Standard Antioxidant Drug.

Statistical analysis

All tests were carried out in triplicates. Data were presented as mean ± SD. To evaluate significant relationships between experimental parameters by correlation and regression analysis were used. Prism Graph Pad 8 and Microsoft Excel 2013 were used for the statistical and graphical evaluations.

RESULTS AND DISCUSSION

Total Antioxidant Capacity (TAC) of Polyherbal Formulation

Phosphomolybdate assay is based on the decrease in Phosphate-Mo (VI) to Phosphate Mo (V) by the sample and subsequent establishment of a bluish-green colored phosphate/Mo (V) complex at acid pH. The phosphomolybdenum technique is regularly applied in the laboratory to assess the total antioxidant capability of plant extracts (Resat Apak, 2016), (Rohan Sharadanand Phatak, 2014).

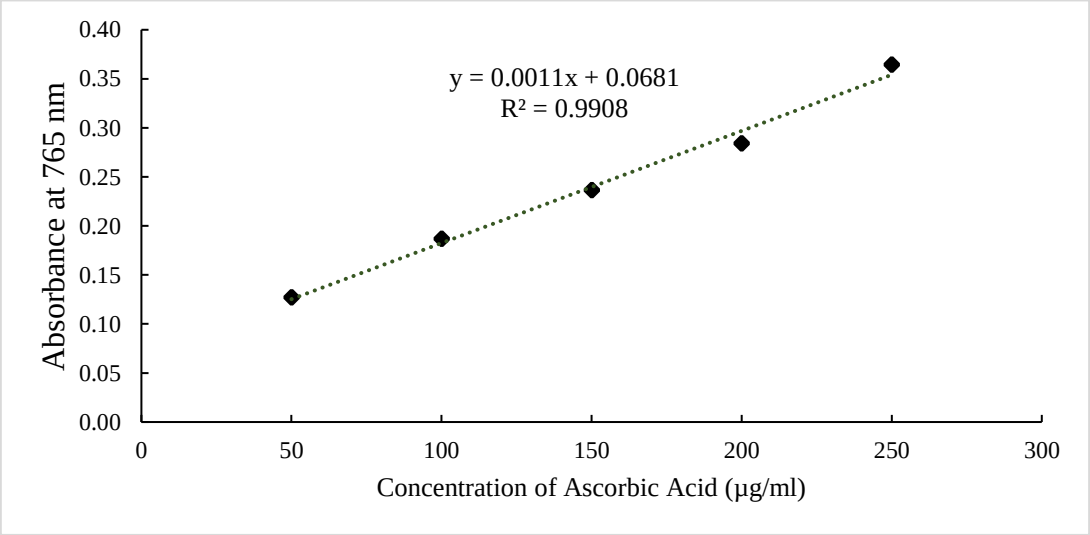

Fig.1: Calibration Curve of Ascorbic Acid

Table 2: Total Antioxidant effect of Polyherbal Formulation

S. No	Conc. µg/ml	Total antioxidant capacity (µg AAE/g)*						
		A. Sativum	*P. Granatum*	*Z. Officinale*	*S. Cumini*	PHF_1	PHF_2	PHF_3
1	50	7.88±1.39	50.61±1.39	20.61±1.39	109.1±9.1	98.79±0.53	109.7±1.9	107.28±0.91
2	100	20.31±1.39	66.37±0.91	22.43±0.53	115.16±5.25	108.79±1.39	124.85±1.39	120.91±1.82
3	150	32.13±1.39	83.04±1.39	27.58±0.53	130.61±0.53	170.91±0.91	196.07±3.79	185.46±0.91
4	200	43.94±1.39	96.37±1.82	29.7±1.39	144.85±0.53	199.4±1.39	220.31±1.39	213.64±1.82
5	250	46.97±0.53	108.19±0.91	33.94±1.39	170.31±1.39	241.22±1.05	268.19±1.82	250.61±1.05

The polyherbal formulation PHF2 shows the highest total antioxidant capacity (TAC) equivalence to Ascorbic acid per gram (µg AAE/g) as compared to polyherbal formulation PHF1 and PHF3. Also, all polyherbal formulations illustrate a significant synergistic impact as compared to individual extracts.

DPPH radical scavenging activity

DPPH is a stable free radical with an absorption band at 515 nm. When reduced by an antioxidant to form DPPH, the natural deep violet color of DPPH modifications to pale yellow. The variation in the color would be proportionate to the power of the antioxidants and a substantial reduction in the absorbance

of the reaction mixture signifies significant free radical scavenging activity of the test material (Dontha, 2016). The impact of antioxidants on DPPH is believed to be owing to their hydrogen donating ability. Radical scavenging activities are extremely important to stop the deleterious role of free radicals in different diseases, comprising diabetes, cancer, and numerous disorders. DPPH free radical scavenging is an accepted method for screening the antioxidant activity of plant extracts. In the DPPH assay, violet color DPPH solution is decreased to a yellow-colored product, diphenylpicryl hydrazine, by the inclusion of the extract in a concentration-dependent manner. This method has been adopted extensively to forecast antioxidant activities because of the fairly short time expected for analysis. Our results elucidated that the polyherbal formulation (PHF2) shows highest free radical scavenging activity with IC50 = 43.57 µg/ml when evaluated with standard Ascorbic acid 48.25 µg/ml, PHF3 62.75 µg/ml and PHF1 88.15 µg/ml (Fig. 2, Table 02).

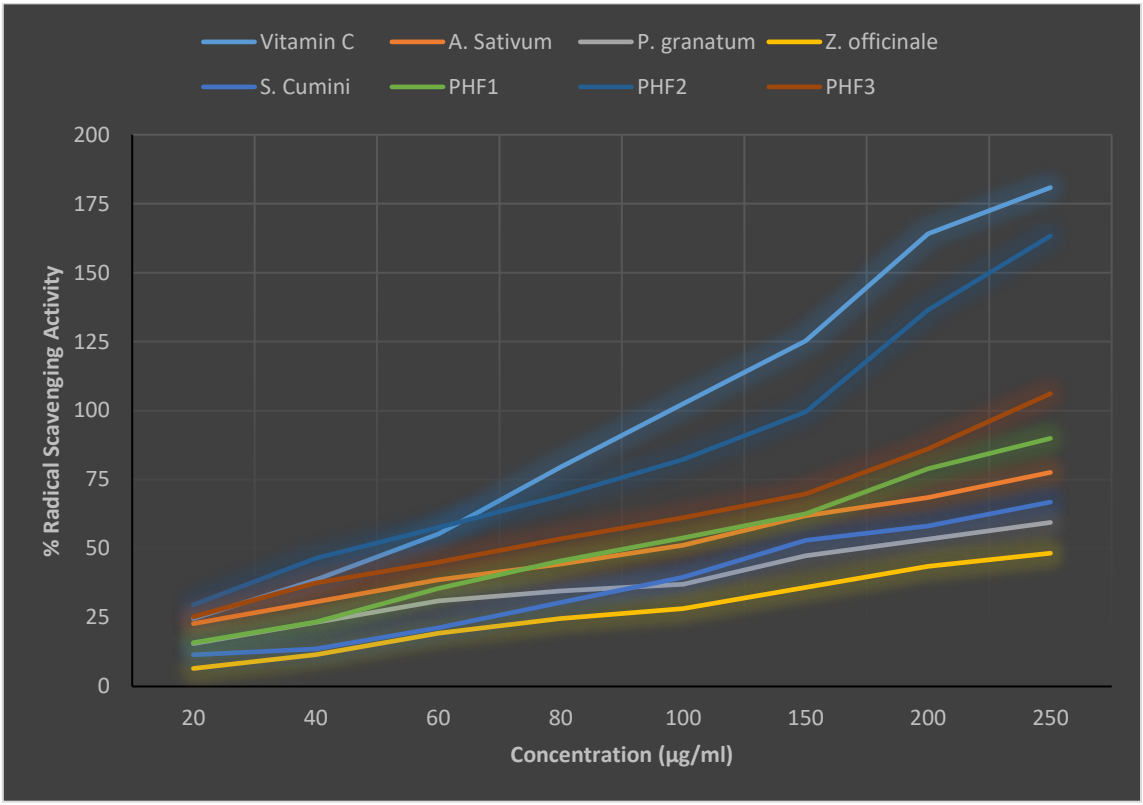

Fig.2: DPPH Radical Scavenging Activity of Polyherbal Formulation

Table 3: Radical Scavenging Activity of Polyherbal Formulation (IC50) on DPPH method

Treatment	**IC50 µg/ml**
Vitamin C	48.25
A. Sativum	90.37
P. granatum	168.7
Z. officinale	261.5
S. Cumini	146.7
PHF1	88.15
PHF2	43.57
PHF3	62.75

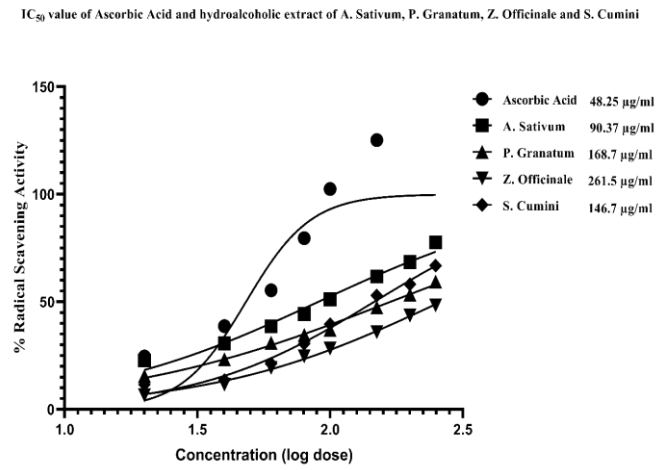

Figure 3a: Determination of IC50 value of Ascorbic Acid and hydroalcoholic extract of *A. Sativum, P. Granatum, Z. Officinale* and *S. Cumini*

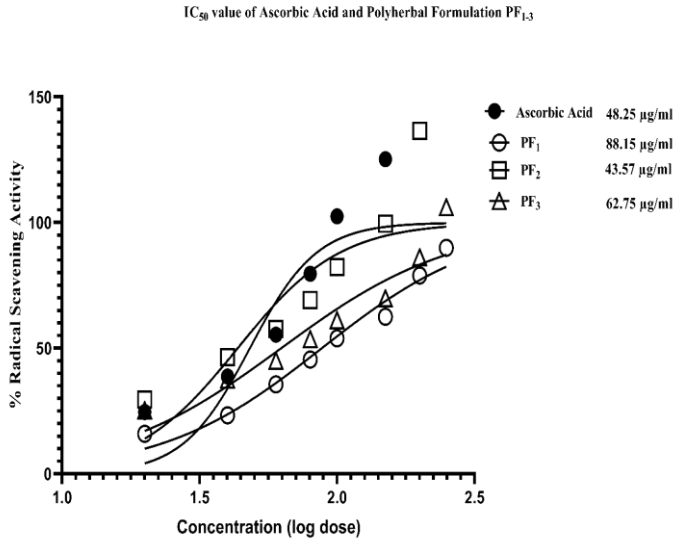

Figure 3b: Determination of IC50 Value of Ascorbic Acid, and Polyherbal Formulation (PHF1-3)

CONCLUSION

The study shows that the evolved polyherbal formulation (PHF2) is effective in significantly reducing the Phosphate-Mo (VI) to Phosphate Mo (V) and illustrates significant DPPH free radical scavenging activity when assessed with standard Ascorbic acid and individual extracts. Hence, the evolved polyherbal formulation might substantiate to be a safe alternative treatment for the management of chronic metabolic disorders like diabetes, cancer, liver injury, inflammation, skin damages, coronary heart diseases, and arthritis through reducing oxidative stress.

ACKNOWLEDGEMENT

The authors wish to thanks to Principal and all management members, Channabasweshwar Pharmacy College (Degree), Latur, Maharashtra for providing the necessary facility to carry out this research work with great ease and precision and Kisalaya Herbals Limited, Indore, Madhya Pradesh, India for providing herbal extract, carryout our research project work.

REFERENCES

1. Atina Rizkiya Choirunnisa, I. F. (2016). Comparison of Five Antioxidant Assays for Estimating Antioxidant Capacity from Three Solanum Sp. Extracts. Asian J Pharm Clin Res, 9(2), 123-128.
2. Chutulo EC, C. R. (2020). Chemical compositions, cytotoxicity and antioxidant activity of the endophytic fungus Fusarium napiforme isolated from Psidium guajava. Studies in Fungi, 5(1), 332-52.
3. Dontha, S. (2016). A Review on Antioxidant Methods. Asian J Pharm Clin Res, 9(2), 14-32.
4. Fereidoon Shahidi, Y. Z. (2015). Measurement of antioxidant activity. Journal of functional foods, 18, 757-81.
5. Garland K. More, R. T. (2020). In-vitro analysis of free radical scavenging activities and suppression of LPS-induced ROS production in macrophage cells by Solanum sisymbriifolium extracts. Scientific Reports, 10(6493), 1-9.
6. Mahbubur Rahman, B. I. (2015). In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. BMC Res Notes, 8(621), 1-9.
7. Maria Agostina Frezzini, F. C. (2019). Application of DPPH Assay for Assessment of Particulate Matter Reducing Properties. Atmosphere, 10(816), 1-14.

8. Mayank Gangwar, M. K. (2014). Antioxidant Capacity and Radical Scavenging Effect of Polyphenol Rich Mallotus philippenensis Fruit Extract on Human Erythrocytes: An In Vitro Study. The Scientific World Journal, 1-12.
9. Rahmat Ali Khan, M. R. (2012). Assessment of flavonoids contents and in vitro antioxidant activity of Launaea procumbens. Chemistry Central Journal, 6(43), 1-11.
10. Renuka Diwan, A. S. (2012). Phytochemical Composition and Antioxidant Potential of Ruta graveolens L. In Vitro Culture Lines. Journal of Botany, 1-6.
11. Resat Apak, ِ. O. (2016). Antioxidant Activity/Capacity Measurement. 1. Classification, Physicochemical Principles, Mechanisms, and Electron Transfer (ET)-Based Assays. J. Agric. Food Chem., 64, 997-1027.
12. Rohan Sharadanand Phatak, A. S. (2014). Total antioxidant capacity (TAC) of fresh leaves of Kalanchoe pinnata. Journal of Pharmacognosy and Phytochemistry, 2(5), 32-35.
13. Shumaila Jan, M. R. (2013). Assessment of Antioxidant Potential, Total Phenolics and Flavonoids of Different Solvent Fractions of Monotheca Buxifolia Fruit. Osong Public Health Res Perspect, 4(5), 246-54.