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Research Article

COMPARATIVE STUDY OF WOUND HEALING ACTIVITY OF INDIAN MEDICINAL PLANT AND HERB AGAINST DIABETIC FOOT ULCER (*PIPER NIGRUM* AND *ARACHIS HYPOGAEA*)

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ABSTRACT

Objective: Diabetic foot ulcers (DFUs) are the most common cause of non-traumatic lower extremity amputations in developing countries. The aim of this pilot study was to evaluate the medicinal plant (fruits) and herbs (seeds) to meet their primary health-care needs healing to DFUs.

Methods: Three solvents were used to acquire extracts from powdered parts of the species. The extracts were used for phytochemical screening like a standard procedure. Quantitative assay of antioxidant activity of the extracts using free radical scavenging phenomena such as a 1,1-diphenyl-2-picrylhydrazyl-assay. The antimicrobial activity of the test organisms to the 11 plant extracts was screened using the agar well diffusion method (Perez *et al*., 1990). Hypotonicity-induced human red blood cell (HRBC) assay performs to determine anti-inflammatory activities.

Results: The presence of maximum antioxidant activity found in *Arachis hypogaea* (ethyl acetate) extract followed by *Piper nigrum*. The comparative study of these plants and herb species (*P. nigrum* and *A. hypogaea*), where *P. nigrum*, contains more total tannin contents and antioxidant compounds as persistent manner compares to *A. hypogaea*. Ethanolic and ethyl acetate, extracts of *P. nigrum* were studied for the *in vitro* antimicrobial and anti-inflammation against DFU contamination and HRBCs. *P. nigrum* treated on DFU contamination and HRBC, shown with maximum inhabitation properties for DFU (800 µg/mL).

Conclusions: The finding of the present investigation demonstrated that *P. nigrum* significantly more suppresses the growth of DFU contamination and induces anti-inflammatory activities follow by *A. hypogaea*.

Keywords: Antioxidant, Diabetic foot ulcer, Anti-inflammation.

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INTRODUCTION

Diabetes mellitus is a disorder of chemical, chemistry due to which multiple long-term complications that affect almost every system in the body. Diabetic foot ulcer (DFU) is a complex pathological phenomenon in which neuropathy and vasculopathy play a key role and is together termed as neuro-ischemic ulcer [1]. Foot ulcers are one of the main complications of diabetes mellitus. Epidemiology of DFU diabetes is a worldwide epidemic; there were more than 230 million individuals with diabetes in 2006. According to consensus, this number is expected to reach 350 million by 2030. Major limb amputation every 30 s, over 2500 limbs lost per day due to diabetes results over the world. One of the diabetic results is DFUs which are a common lower extremity complications of diabetes. Foot ulcers, therefore, play a central role in the causal pathway to lower extremity amputation [2]. In general, diabetics with ulcers commonly experience infection with Gram-positive organisms such as Staphylococcus aureus, and Enterococcus, and Gram-negative organisms such as Pseudomonas aeruginosa, Escherichia coli, Klebsiella species, and Proteus species, and anaerobes [3].

Spices and herbs of Indian origin are known for their pungent aroma and delightful flavor and used indispensably in culinary preparations [4]. *Piper nigrum* (black pepper), family Piperaceae, considered as "the king of spices'' among various spices. It contains major pungent alkaloid piperine (1-peperoyl piperidine), which exhibits diverse pharmacological activities such as antihypertensive and antiplatelets, antioxidant, antitumor, antiasthmatics, antipyretic, analgesic, anti-inflammatory, anti-diarrheal, antispasmodic, anxiolytic, antidepressants, hepato-protective, immuno-modulatory, antibacterial, antifungal, anti-thyroids, antiapoptotic, anti-metastatic, antimutagenic, antispermatogenic, anticolon toxin, insecticidal, and larvicidal activities [5]. Peanut (*Arachis hypogaea*), also known as groundnut, earthnut, or goober, legume of the pea family (*Fabaceae*), grown for its edible seeds. There are thousand verities of peanut cultivars around the world. Certain groups of cultivars are preferred for particular uses because of differences in flavor, oil content, size, shape, and disease resistance. The most popular cultivars are Spanish, Runner, Virginia, and Valencia. China leads in production of peanuts, having a share of about 45% of overall world production, whereas India has (16%) share and the United States of America has (5%) [6].

Naturally occurring elements in complex plants have antioxidant activity. Recently, there is a growing interest in oxygen-containing, free radicals are highly unstable molecules that are naturally formed when you exercise and when your body converts food into energy. Triggering of the cell damage caused by the free radicals result of oxidative stress is thought to play a role in a variety of diseases including cancer, cardiovascular diseases, diabetes, Alzheimer's disease, Parkinson's disease, and eye diseases such as cataracts and age-related macular degeneration [7]. The present study is aimed at elucidation of antioxidant activity, antimicrobial activity for DFU, anti-inflammation, and characterization of the active principles from the extracts of the fruits of *P. nigrum* and seeds of *A. hypogaea*.

Polyphenols and tannins are water-soluble that is commonly found in higher herbaceous and woody plants. They can be classified into two categories: Hydrolysable and non-hydrolysable (condensed) [8]. Phenolic compounds are a unique category of plant phytochemicals especially in terms of their vast potential health-benefiting properties. Tannin has been considered to be cardio-protective, anti-inflammatory, anti-carcinogenic, and anti-mutagenic, among others. Tannins compounds enhance glucose uptake and inhibit adipogenesis, thus

being potential drugs for the treatment of non-insulin-dependent diabetes mellitus. Tannins can improve the pathological oxidative state of a diabetic situation [9]. The phenols are dietary antioxidants include ascorbate, tocopherols, carotenoids, and bioactive plant phenols. The health benefits of fruits and seeds are largely due to the antioxidant biomolecules supported by a large number of phytochemicals, some with greater antioxidant properties. Plant phenols have not been completely studied because of the complexity of their chemical nature and the extended occurrence in plant materials [10]. The present work is to provide an overview of the findings related to the presence of antioxidant, phenols and tannin in plant and herb sources. The antimicrobial and HRBC assay performed on DFUs Sample. Certain groups of researchers have focused on the investigation of plants and microbial extracts, essential oils, pure secondary metabolites, and new synthesized molecules as potential antimicrobial agents. The in vitro antimicrobial activity of extracts or a pure compounds used by most common basic methods are the disc diffusion and broth or agar dilution methods. Other methods are used especially for antifungal testing, such as poisoned food technique. It is important to develop a better understanding of the current methods available for screening and/or quantifying the antimicrobial effect of an extract or a pure compound for its applications in human health, agriculture, and environment. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute for bacteria and yeast testing [11].

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: The increase of vascular permeability, increase of protein denaturation, and membrane alteration [12]. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form stress. The inflammation of tissue is due to response to stress [13]. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. The real issue in the rural community to management of inflammation related diseases and the population in these areas uses many alternative drugs such as substances produced from medicinal plants [12].

Table 1: Qualitative phytochemical analysis of *Piper nigrum*

S. No.	Phytochemical/solvents	Hexane	Ethyl acetate	Ethanol
1.	Tannin	+	++	+
2.	Saponin	+	+	–
3.	Flavonoids	–	+	+
4.	Alkaloids	–	–	+
5.	Quinones	+	++	–
6.	Glycosides	–	–	–
7.	Cardioglycosides	+	+	++
8.	Terpenoids	+	++	+
9.	Phenols	–	+	++
10.	Coumarins	–	+	+
11.	Steroids	++	++	+
12.	Phlobatannins	–	–	–
13.	Anthraquinones	–	–	–

++: Highly present, +: Moderately present, –i Absent

Table 2: Qualitative phytochemical analysis of *Arachis hypogaea*

S. No.	Phytochemical/solvents	Hexane	Ethyl acetate	Ethanol
1.	Tannin	–	+	++
2.	Saponin	+	+	+
3.	Flavonoids	–	–	–
4.	Alkaloids	+	+	+
5.	Quinones	–	–	+
6.	Glycosides	–	–	–
7.	Cardioglycosides	+	+	++
8.	Terpenoids	+	+	+
9.	Phenols	+	++	++
10.	Coumarins	–	–	–
11.	Steroids	+	+	+
12.	Phlobatannins	–	–	–
13.	Anthraquinones	–	–	–

++: Highly present, +: Moderately present, –: Absent

METHODS

Preparation of powder

The *P. nigrum* (100 g) and *A. hypogaea* (100 g) washed with fresh water to remove adhering dust and then dried under shade for 2 days. After shade dried, for estimation of powder was using a mixer grinder and in turn extracted with different solvents, namely, hexane (non-polar), ethyl acetate (polar), and 75% ethanol (strong polar). The extraction mixture was left untouched for a day before the extracted was separated from the residue by filtration through Whatman No 1 filter paper. These extraction samples collected into jar and left its for evaporation for days.

Phytochemical screening

The extracts are tested for the presence of bioactive compounds (tannin, saponin, flavonoids, quinine, glycoside, cardiac glycoside, terpenoid, phenol, coumarins, steroid, alkaloids, phlobatannins, and anthraquinones) using followed by phytochemical screening standard methods.

Quantitative assay of antioxidant activity

Leaf extract samples of 100 µl from qualitative assay were mixed with 2.7 ml of methanol. Then, 200 µl of 0.1% methanolic 1,1-diphenyl-2-picrylhydrazyl was added. The suspension was incubated for 30 min in dark conditions. Eventually at every 5 min interval, the absorption maxima of the solution was measured using an ultraviolet (UV) double beam spectra at 517 nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% of butylated hydroxytoluene.

The radical of sample is calculated by the following formula

$$\text{Inhibition\%} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

Quantitative phytochemical analysis

Determination of tannin content

The tannin content in the extracts was determined by Folin-Ciocalteu method. 0.1 ml of the sample extracts containing 1 mg were added to test tubes containing 7.5 ml of distilled water and 0.5 ml of Folin-

Table 3: Total tannin contains in *Arachis hypogaea* and *Piper nigrum*

S. No.	Sample/g	Total tannin contents concentration (mg tannic acid/g dry sample)
1.	*Piper nigrum*	109.75
2.	*Arachis hypogaea*	75.85

Table 4: Total phenol contains in *Arachis hypogaea* and *Piper nigrum*

S. No.	Sample/g	Total phenol contents concentration (mg GAE/g dry sample)
1.	*Piper nigrum*	122.4
2.	*Arachis hypogaea*	175.7

Table 5: Formula of respective solvents and antimicrobial

Hexane	C_6H_{14}	Solvent
Ethyl acetate	$C_4H_8O_2$	Solvent
Ethanol	C_2H_5OH	Solvent
Tetracycline	$C_{22}H_{24}N_2O_8$	Antimicrobial

Ciocalteu reagent, 1 ml of 35% Na_2CO_3 and made it up to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of tannic acid (20, 40, 60, 80, and 100 µg/ml) was also used. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid equivalence (TE) µg/mg of extract.

Determination of total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu method [14]. Total phenolic content was determined by the Folin-Ciocalteu method use the gallic acid calibration curve for quantification of total polyphenols(14). From the standard solution 20–100 µl was taken and added to different test tubes. The extract was added in a separate test tube at a concentration of 10 mg/ml, and 5ml of Folin-Ciocalteu (1:10 dilution) was added, and the contents were mixed thoroughly. 4 ml of 0.7 M sodium carbonate was added, and the mixture was incubated for 30 min. The absorbance was determined at 765 nm in a UV-visible spectrophotometer. The results were expressed in gallic acid equivalence of the samples (GE) µg/mg of the extract.

Table 6: The diameters of inhibition growth zones of samples extract with different solvent and antimicrobial agents (control) are measured in mm

Arachis hypogaea	**Extract**	**Inhibition (mm)**	**Inhibition (mm)**
Biotic	**Sample (µl)**	**Sample**	**Control ($C_{22}H_{24}N_2O_8$)**
DFU (C_6H_{14})	20	1	15
DFU (C_6H_{14})	40	4	15
DFU (C_6H_{14})	60	4.6	15
DFU (C_6H_{14})	80	8.2	15
DFU ($C_4H_8O_2$)	20	2	14
DFU ($C_4H_8O_2$)	40	3	14
DFU ($C_4H_8O_2$)	60	6.2	14
DFU ($C_4H_8O_2$)	80	10	14
DFU (C_2H_5OH)	20	-	10
DFU (C_2H_5OH)	40	-	10
DFU (C_2H_5OH)	60	-	10
DFU (C_2H_5OH)	80	-	10
Piper nigrum	**Extract**	**Inhibition (mm)**	**Inhibition (mm)**
Biotic	**Sample (µl)**	**Sample**	**Control ($C_{22}H_{24}N_2O_8$)**
DFU (C_6H_{14})	20	2	15
DFU (C_6H_{14})	40	5	15
DFU (C_6H_{14})	60	8	15
DFU (C_6H_{14})	80	10	15
DFU ($C_4H_8O_2$)	20	1	14
DFU ($C_4H_8O_2$)	40	-	14
DFU ($C_4H_8O_2$)	60	-	14
DFU ($C_4H_8O_2$)	80	-	14
DFU (C_2H_5OH)	20	3	10
DFU (C_2H_5OH)	40	5	10
DFU (C_2H_5OH)	60	8	10
DFU (C_2H_5OH)	80	11	10

Antimicrobial activity screening tests

The antimicrobial activity of the test organisms to the 11 plant extracts was screened by using the agar well diffusion method (Perez *et al*., 1990). Swabbed Uniformly of suspension inoculum on solidified 20 mL Mueller-Hinton Agar for bacteria and Sabouraud dextrose agar for fungi and the inoculum was allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar using sterile cork borer. Take 50 µL of aliquot from each plant crude extract (500 mg/mL) was added into each well on the seeded medium and allowed to stand on the bench for 60 min for proper diffusion and thereafter incubated at 37°C for 24 h. The resulting inhibition zones were measured in millimeters. The same procedure was followed for the fungus *Candida albicans* but incubated at 30°C. Negative controls using 50 µL tetracycline were also run in the same manner and parallel to the treatments. These studies were performed in triplicate. The minimal microbial concentration and minimum inhibitory concentration were determined for the active plant extracts that showed the widest spectrum of antimicrobial activity against test microorganisms.

Hypotonicity-induced human red blood cell (HRBC)

1.0 mL of test sample of different concentrations (50–200 µg) in 1 ml of 0.2 M phosphate buffer and 0.5 mL of 10% HRBC suspension incubated at 37°C for 30 min, with 0.5 ml of 0.25% hyposaline and centrifuged at 3000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac was used as standard and control was prepared by distilled water instead of hypo saline to produce 100% hemolysis without samples. The percentage of HRBC hemolysis and membrane stabilization or protection was calculated using the following formula:

Percentage of hemolysis = (Optical density of test sample/optical density of control)×100

Percentage protection = 100−([Optical density of test sample/optical density of control]×100) [15].

RESULTS AND DISCUSSION

Qualitative phytochemical analysis of *P. nigrum* and *A. hypogaea*, fruits, and seeds extract

The phytochemical screening of the plant studied showed the presence of tannin, saponin, flavonoids, quinine, glycoside, cardiac glycoside,

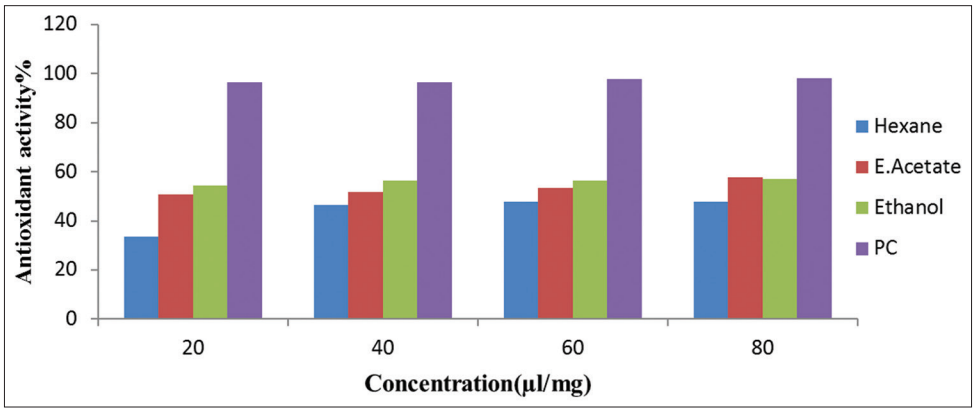

Fig. 1: 1,1-diphenyl-2-picrylhydrazyl free radical scavenging percentage of A*rachis hypogaea*

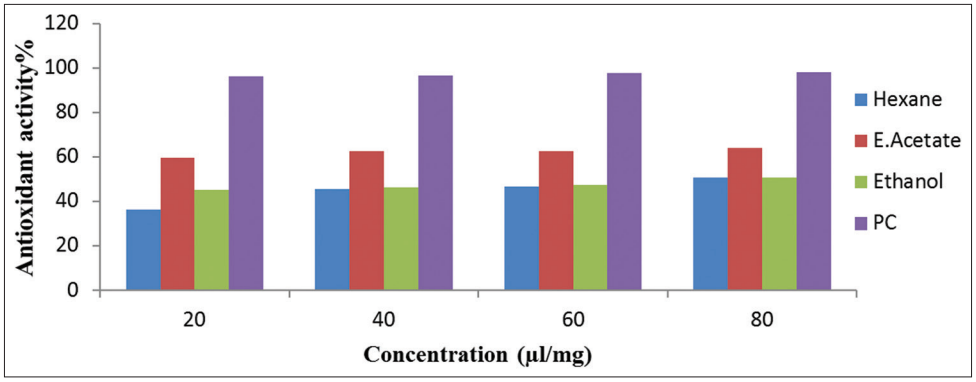

Fig. 2: 1,1-diphenyl-2-picrylhydrazyl free radical scavenging percentage of *Piper nigrum*

Table 7: Percentage hemolysis activities of sample black pepper and positive control

Sample	% hemolysis	
Concentration (µg)	Sample black pepper (ethyl acetate extract)	PC (diclofenac)
50	16.40	16.68
150	14.35	12.10
200	8.20	8.82

Table 7.1: Percentage protection activities of samples and positive control

Sample	% protection	
Concentration (µg)	Sample black pepper (ethyl acetate extract)	PC (diclofenac)
50	83.6	83.32
150	85.65	87.90
200	91.8	91.18

Table 8: Percentage hemolysis activities of sample peanut and positive control

Sample	% hemolysis	
Concentration (µg)	Sample peanut (ethanol extract)	PC (diclofenac)
50	30.35	16.68
150	27.48	12.10
200	22.35	8.82

Table 8.1: Percentage protection activities of samples and positive control

Sample	% protection	
Concentration (µg)	Sample peanut (ethanol extract)	PC (diclofenac)
50	69.65	83.32
150	72.52	87.90
200	77.65	91.18

terpenoids, phenol, coumarins, steroids, alkaloids, phlobatannins, and anthraquinones. On *P. nigrum* (Table 1) ethyl acetate fruits extract was found the highest positive response followed by other solvents while in *A. hypogaea* (Table 2) ethanol seeds extract was found the highest positive response followed by other solvents such as hexane and ethyl acetate of fruits and seed extracts, respectively.

Quantitative phytochemical screening

The tannin content was expressed in terms of mg of TE µg/mg of extract and GE of the samples (GE) µg/mg of the extract. While *P. nigrum* exhibited the maximum content of Tannin (Table 3), whereas *A. hypogaea* exhibited the maximum content of phenol (Table 4) compounds comparatively together, hence greatest antioxidant profiles these two compounds of plant and herb contains.

In vitro antioxidant activity: Percentage: Among these, two species of herb and plant are used for maximum antioxidant activity found in *A. hypogaea* (ethyl acetate: Fig. 1) extract followed by *Piper nigrum* (Fig. 2). Table 5 *shows* formula of respective solvents and antimicrobial.

We found that in this study, the plant and herb extracts by ethanol and ethyl acetate of *P. nigrum* and *A. hypogaea,* respectively, provided more consistent antimicrobial activities for DFU contaminations, compared to those extracted by other solvents (Table 6).

Antimicrobial assay

The inhibition zones of the Antimicrobial assay were measured in millimetres shown in table 6. The Arachis hypogaea and the Piper nigrum, crude extract of samples with ($C_4H_8O_2$) and (C_2H_5OH) respectively shown maximum antimicrobial activities.

Hypotonicity-induced human red blood cell membrane stabilization method

This above mention table percentage of hemolysis and protection is shown that ethyl acetate extract of P. nigrum (Tables 7 and 7.1) has anti-inflammatory properties more comparatively *A. hypogaea* (Table 8 and 8.1) and can reduce inflammatory injury and tissue damage.

CONCLUSION

The study clearly indicates that the extract possesses antioxidant, antimicrobial, and anti-inflammatory substances. At the specific space and time of abiotic factors induces more ability to suppress growths of DFU contamination and anti-inflammatory activities comparison to underground abiotic factors.

These findings justify the traditional uses of this plant's fruit in the treatment of DFU, inflammatory.

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AUTHORS' CONTRIBUTIONS

Dr. Sharmila KJ, supervised my project work and reviewed the manuscript; and Dr. R. Caroline Jeba provided the chemical details and the samples.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Karri VV. Current perspective in the management of diabetic foot ulcers an overview on the Indian scenario. Int J Pharm Pharm Sci 2014;6:1-2.
2. Mariam TG, Alemayehu A, Tesfaye E, Mequannt W, Temesgen K, Yetwale F, *et al.* Prevalence of diabetic foot ulcer and associated factors among adult diabetic patients who attend the diabetic follow-up clinic at the university of gondar referral hospital, North West Ethiopia, 2016: Institutional-based cross-sectional study. J Diabetes Res 2017;2017:2879249.
3. Banu A, Noorul Hassan MM, Rajkumar J, Srinivasa S. Spectrum of bacteria associated with diabetic foot ulcer and biofilm formation: A prospective study. Australas Med J 2015;8:280-5.
4. Gupta D, Girija K. Evaluation of *in vitro* antioxidant and anti microbial activities of various spices of Indian origin. Int J Pharm Pharm Sci 2015;7:137-41.
5. Damanhouri ZA, Ahmad A. A review on therapeutic potential of *Piper nigrum* L. (Black Pepper): The king of spices. Med Aromat Plants 2014;3:161.
6. Arya SS, Salve AR, Chauhan S. Peanuts as functional food: A review. J Food Sci Technol 2016;53:31-41.
7. Kumar SR, Sridhar C, Jayaveera KN. A study on total phenols, flavonoids content and antioxidant activity for the leaf extracts of *Holostemma ada kodien* schult traditionally used folklore medicinal plants. Indo Am J Pharm Res 2013;4:1499-512.
8. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against Staphylococcus aureus. J Antimicrob Chemother 2001;48:487-91.
9. Kumari M, Jain S. Tannin: An antinutrient with positive effect to manage diabetes. Res J Recent Sci 2012;1:1-8.
10. Dimitrios B. Sources of Natural Phenolic Antioxidants. Laboratory of Food Chemistry and Technology, School of Chemistry, Aristotle University. Thessaloniki: Elsevier Ltd.; 2006.
11. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm Anal 2016;6:71-9.
12. Leelaprakash G, Dass SM. *In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*. Int J Drug Dev Res 2011;3:189-96.
13. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, *et al.* Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2018;9:7204-18.
14. Slinkard K, Singleton VL. Total phenol analysis: Automation and comparison with manual methods. Am J Enol Viticult 1997;28:49-55.
15. Somani Z, Bhattacharjee R, Chodankar M, Abhilash N. *In vitro* antioxidant, anti-inflammatory, inovo anti-angiogenic activities virtual screening of phyto constituents of chromolenaodorata. Int J Curr Res 2014;6:7766-71.