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ANTIOXIDANT, HEMOLYTIC AND MUTAGENIC POTENTIAL OF *PSORALEA CORYLIFOLIA*

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ABSTRACT

Plants are one of the natural sources of bioactive molecules with potential abilities against different stress conditions. During pathogenic exposure, an array of biologically active molecules like reactive oxygen intermediates, phytoalexins and defense peptides are synthesized as measure of plant resistance to combat the intensifying conditions. Such molecules play a key role in advancement in potential therapeutics development. *Psoralea corylifolia (P. corylifolia)*, a medicinal herb was evaluated under fungal stress and normal conditions for its inherent bioactive potential. Under fungal stress plant protein/peptide extracts showed greater antioxidant potential evaluated by DPPH radical scavenging activity assay. Mutagenicity of the extracts showed that the plant was safe to use. Hemolytic activity assay exhibited increased percentage in induced seedling extracts that instigates possible applications of extracts against cancer.

Keywords: Bioactive molecules, pathogenic, *P. corylifolia*, bioactive potential, proteins/peptides.

INTRODUCTION

Recently, health concerns and toxicology of synthetic antioxidants have intensified the study for edible plants exhibiting effective, natural and non-toxic antioxidant compounds (Iqbal *et al.* (2012). Antioxidants act as repository of anti-inflammatory, antifungal, antibacterial and anti-carcinogenic (Servili *et al.* (2014). Reactive oxygen species (ROS), being one of the most rapid defense reactions induces production of antioxidant compounds. Consumption of these antioxidants from plants appears to be a healing realm against oxidative burst induced degenerative and pathological diseases like ageing and cancer (Pontiki *et al.* 2014; Tadhani *et al.* 2007).

Plants rely on proteomic pliability and bioactive molecules to amend themselves during pathogenic exposure. In response to microbial stress, crucial activities like induction and expression of genome-wide defensin genes, production of antioxidants and other bioactive molecules take place (Falak and Jamil, 2013). Pursuit for potential therapeutic agents especially under stress conditions is all muse on evaluating medicinal plants. Also mutagenicity and toxicity analysis at cellular level is necessary to develop safe measures of phyto-medicines.

In view of the above facts *Psoralea corylifolia* (leguminosae, Babchi) selected for evaluation of possible antioxidant, mutagenic and cytotoxic potential under biotic stress. Numerous studies report coumarins and flavonoids to be the major bioactive constituents of fructus psoralea (Chopra *et al.* (2013). *Psoralea corylifolia* has wide applications in ayurvedic and traditional Chinese medicine due to its antioxidant, antitumor, antibacterial, antifungal, immunomodulatory and anti-inflammatory activity (Gidwani *et al.* (2011); Qiao *et al.* (2006). Strong antioxidant activity in *P. corylifolia* fruit extract is reported in ABTS (2, 2V-azinobis[3-ethylbenzothiazoline-6-sulfonate] assay (Tang *et al.*(2004). Psoralen, isopsoralen, bakuchiol, corylifolin, corylin and especially psoralidin from powder, ether and chloroform extract of *P. corylifolia* showed strong antioxidant potential in comparison to butylated hydroxyl toluene and a-tocopherol (Jiangning *et al.* (2005). In addition, this plant species possesses a wide range of biological activities like cytotoxicity, antimutagenic activity and bone calcification (Sah *et al.* (2006). Study on insecticidal and genotoxic activity of the plant is also reported while no literature was found on mutagenic and hemolytic activity of *P. corylifolia.*

In this study, fungal stress enhanced the antioxidant and hemolytic activity of *Psoralea corylifolia* seedlings while no mutagenic activity was observed in normal and fungal stress conditions.

MATERIALS AND METHODS

Plant material: Seeds of *Psoralea corylifolia* were collected from local market in Faisalabad and authenticated from Department of Botany, University of Agriculture, Faisalabad, Pakistan. The seeds were sterilized and germinated in Petri plates (Falak and Jamil, 2013).

Crude protein extraction from *Psoralea corylifolia* seeds and seedlings: The seeds and seedlings of *P. corylifolia* were ground to a fine powder in pestle and

mortar under liquid nitrogen and weighed upto 5 g each in sterilized centrifuge tube. The samples were homogenized in15µl of sodium phosphate buffer and 150 µl of 1 mM PMSF (phenylmethylsulfonyl fluoride) on polytrone for 15 minutes with 30 second intervals. The samples were centrifuged for 20 minutes at 10,000 rpm and 4 °C. Supernatant having crude extract was collected and stored at -20 °C for further study (Falak and Jamil, 2013).

Fungal induction: *Fusarium solani* was used to induce stress in *P. corylifolia* seedlings. Inoculum of *Fusarium solani* was prepared in Saboraud's medium (Oxoid) as described by Singariya *et al.* (2012) in 100 mL of distilled water and pH 5.7 was maintained. The inoculum was autoclaved for 15 minutes at 121°C. On cooling it was inoculated with *F. solani* spores and incubated at 28°C at 120 rpm for three days. *Psoralea corylifolia* seedlings were induced with 1×10^5 spores/ mL of the fungal culture and harvested along with their control samples 8 h post inoculation. The samples were preserved in liquid nitrogen and stored at -80°C freezer till further use (Falak and Jamil, 2013).

Protein isolation: The fungus-induced seedlings were ground to a fine powder in liquid nitrogen without thawing and protein extraction was performed by following the TCA/acetone precipitation/phenol method described by Rastegari *et al.* (2011). These extracts were then further used for evaluation of antioxidant, hemolytic and mutagenic potential.

Antioxidant activity assay: DPPH % RSA method was followed after Noor *et al.* (2014). The protein samples (crude and induced protein extracts) were mixed with DPPH solution in 2:1 proportion followed by incubation for 15 min at room temperature. The absorbance was noted at 517 nm against ascorbic acid as standard. Each experiment was done in triplicate.

Hemolytic activity assay: Hemolytic activity of *P. corylifolia* extracts was conducted by following the method used by Shahid *et al.* (2013) and Zuber *et al.* (2014). After consent and counseling with volunteers 3 mL of freshly heparinized human blood sample was collected and centrifuged at 1000x*g* for 5 min. Plasma was discarded and 5 mL of autoclaved and chilled (4°C) phosphate-buffered saline (PBS) with pH 7.4 was used thrice for washing cells. Each assay was performed with maintained erythrocytes concentration of 10^8 cells per mL.100µL of each extract was mixed with 10^8cells/mL human blood cells separately following incubation for 35 min at 37° C and inverted after 10 min. The samples were immediately transferred on ice after incubation for 5 min and centrifuged at 1000x*g* for 5 min. 100 µL of supernatant was collected from each tube and 10 times diluted with chilled PBS. PBS was taken as negative control whileTriton X-100 (0.1% v/v) was taken as positive control and same procedure was followed. The absorbance was taken at 576 nm using µQuant (Bioteck, USA). The % hemolysis for each sample was calculated.

Mutagenic activity test: Mutagenic activity was evaluated according to the procedure described by Razak *et al.* (2007). Mutagenic activity of *P. corylifolia* extracts was evaluated by the Muta-Chromplate kit (Environmental Bio-detection Products Incorporation (EBPI, Ontario, Canada)) according to manufacturer's protocol. This test kit works on the principle of Ames bacterial reverse-mutation test (Ames *et al*., 1975) performed in liquid culture (fluctuation test). Two mutant bacterial strains *S. Typhimurium* TA98 and *S. typhimurium*TA100 were maintained on nutrient agar at 3 ± 1ºC. The inoculum was prepared by inoculating bacterial strains into nutrient broth and incubated them for 18 - 24 h at 37 ºC.

Mutagenic activity assay: Plant extract, reagent mixture, deionized water, S9 activation mixture and standard mutagen were mixed in different bottles as shown in Table 1 and overnight culture of *S. typhimurium* test strains were used for inoculation. Each inoculated contents from bottles was added into 96-well microtitration plate and incubated for 4 days at 37 ºC.

Interpretation of results and statistical analysis: Positive results are shown by yellow color wells while negative results are indicated by purple color wells. Background plate was prepared for each test strain of *salmonella* to compare its natural rate of reverse mutation (fig 3). The extract with significantly higher number of yellow wells in test plate in contrast to yellow wells in the 'background' plate was considered mutagenic as shown in fig. 3. Positive controls were also prepared for comparison of results.

RESULTS AND DISCUSSION

Potential microbial resistance and fewer side effects of herbal extracts have led to focus on novel therapeutic agents from plants. Advancement in scientific techniques have revealed the complex chemical composition of plants and revaluated the phytomedicine industrial applications. *Psoralea corylifolia* being part of Chinese pharmacopoeia and ayurvedic medicine has been used to treat different diseases and skin disorders (Chopra *et al.* (2013). However, to the best of our knowledge no studies to date have been conducted to compare protein extracts in aqueous phosphate buffer under fungal stress.

In our study, the bioactive potential of crude protein extracts of control and *Fusarium solani* induced seeds and seedlings of *Psoralea corylifolia* were evaluated.

Antioxidant activity: Antioxidant activity is the ability to scavenge free radicals in organisms. Isoflavones from plants are reported to possess antioxidant activity with protective effect against oxidative damage (Devi *et al.* (2009).

Antioxidant potential of *P. corylifolia* was studied in crude extracts of control and *Fusarium solani* induced seedling extracts. All experimental samples showed significant antioxidant activity (Figure 1). Seed crude extract possessed more activity than seedling crude while 8 h induced extract showed greater activity than all protein extracts. Seed and seedling crude extracts showed less antioxidant activity than 8 h induced extract as compared to ascorbic acid (positive control). Maximum antioxidant activity of 62% was possessed by 8 h induced protein extract.

Many phytochemicals including psoralen, isopsoralen, psoralidin, corylifol and bavachinin were reported to possess antioxidant activity form *P. corylifolia.* Bhawya and Anilakumar (2011) studied different solvent extracts of *P. corylifolia* and reported to possess strong % DPPH radical scavenging activity. Water extract showed 40.42% which is in agreement to our results while maximum activity was shown by ethanol extract 91.0%. Methanolic, ethyl acetate, chloroform, hexane and acetone extracts showed 76.42%, 61.27%, 46.24%, 65.93% and 31.05%, respectively. Xiao *et al.* (2010) isolated antioxidant components from *P. corylifolia* seeds by using a combination of thin layer chromatography (TLC) and high-speed counter-current chromatography (HSCCC). Separation by two phases HSSC produced 3.19 mg psoralidin, 5.91 mg psoralen, 6.26 mg isopsoralen, 2.43 mg bavachinin and 0.92 mg corylifol A with maximum purity. Also aqueous and solvent extracts of *P. corylifolia* seeds were also reported to possess superoxide anion quenching. Contrary to our results, maximum superoxide quenching activity (89%) was shown by alcohol and water (1:1) extract (AWEP) (Kiran and Raveesha (2010). Jiangning *et al.* (2005) also studied antioxidant activity of powder and extracts of *P. corylifolia*. Bakuchiol, corylin and corylifolin were found responsible for strong antioxidant activity and psoralidin showed stronger antioxidant potential than BHT, while psoralen and isopsoralen possessed no antioxidant activity at 0.02% and 0.04%.

It is concluded from the results in Figure 1 that 8 h induced protein extract of *P. corylifolia* showed significant antioxidant activity which might be due to induction and synthesis of some proteins for defense purpose in addition to antioxidant compounds like bakuchiol, corylin, corylifolin and psoralidin. Therefore, fungal stress enhanced antioxidant activity of the seedlings as compared to other plant extracts (Figure. 1) which assures efficient use of plant in the treatment of oxidative stress and related diseases.

Hemolytic activity: Hemolytic activity of seeds and seedling crude extracts were analyzed against human erythrocytes. The results showed that hemolytic activity of seed crude and seedling crude extracts was non-significant (Figure 2). In comparison, seed crude extract showed greater activity (8.51%) than the seedling crude extract (5.69%). However, according to results, 8 h induced protein extracts showed greater hemolytic activity of 9.33% among all extracts.

Radiation-induced hemolysis of human erythrocytes by IBG-RA-26 extract of *Psoralea corylifolia* was studied with efficacy in 25-1000 µg/mL concentration range. Significant protection against radiation was observed in 25-50 µg/mL range (Arora *et al.,* 2011). Other plants like *Achyranthes aspera* are reported to possess very low haemolytic activity to human erythrocytes (1.3%) (Priya *et al.,* 2010). Moderate hemolytic activity was found in aqueous extract of *Lantana camara* human erythrocytes with 7.93% at 1000 µg/mL concentration (Kalita *et al.,* 2011).

From our results, significant increase in hemolytic activity of 8 h induced protein extract as compared to 8 h control might be due to increased synthesis of some protein against pathogens possessing hemolytic property. Less hemolytic potential of seed and seedling crude extract as shown in figure 2 makes it safe for optimization of the extracts in pharmacological uses. Overall, all extracts possess less to moderate hemolytic activity which supports the use of the herb in safe measures.

Mutagenic activity: Mutagenic activity investigation of seed and seedling crude extracts was performed against two test strains of *Salmonella* TA98 and TA100 for evaluation of their potential ability to cause cancer. All test samples were found to be non-mutagenic as shown in Table 2.

Mutagenic activity was evaluated by Fluctuation method entirely performed in liquid culture is based on Ames test used for detection of carcinogenicity and gene mutation level (Lewis *et al.,* 1993). The mechanism involved in this test requires mutant strains of *Salmonella typhimurium* which require histidine for growth. The chemical substances like carcinogens have ability to revert to state in which bacterium does not require histidine for growth. The frequency of mutation was measured by pH indicator that cause color changes from purple to yellow. The changes in color of sample are compared to natural background rate activity. This is first time to study the mutagenic potential of *P. corylifolia* with and without stress (fungal).

To the best of our knowledge no studies have been found regarding mutagenic ability of *Psoralea corylifolia* while antimutagenic activity is reported in the respective plant. Quinone reductase (QR) enzyme induced by chemical substances possesses protective

effect against mutagens and carcinogens. Lee *et al.* (2009) studied the effect of seed extracts of *P. croylifolia* on induced QR enzyme in Hepa 1c1c7 hepatoma cells. Methanolic extract of *P. croylifolia* seeds showed 1.5 fold (1.5 FIC) increased QR induction at 1.2 µg/mL concentrations while psoralidin isolated further from active fraction showed 1.5 FIC at 0.5 µg/mL concentrations. Also essential oil from plant is reported to cause genotoxic effects of 6.713% and 8.864% in adult *Cx. quinquefasciatus* at 0.034 mg/cm^2 and 0.069 mg/cm^2 revealed by comet assay method (Dua *et al.*, 2013).

From mutagenic screening of *P. corylifolia* extracts during the current study, it was found that they are non-mutagenic which assures their safety to use in pharmacological formulations.

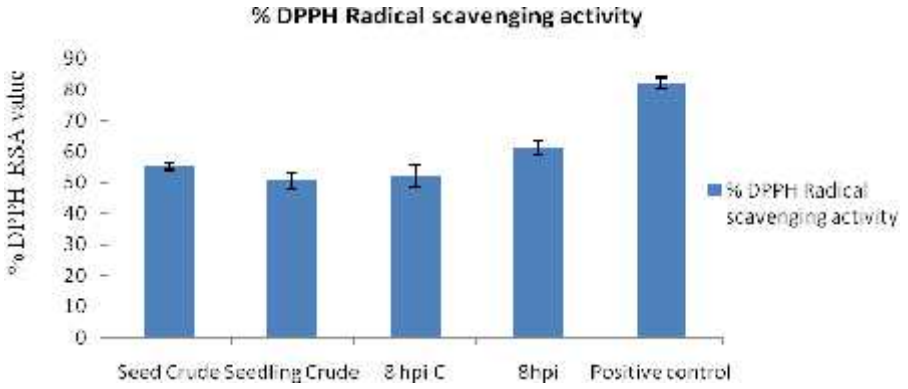

Fig. 1.Antioxidant activity of protein extracts of *P. corylifolia*. Each bar is determinant of mean of three readings. hpi: hours post induction, hpiC: hours post induction control, positive control: Catachin.

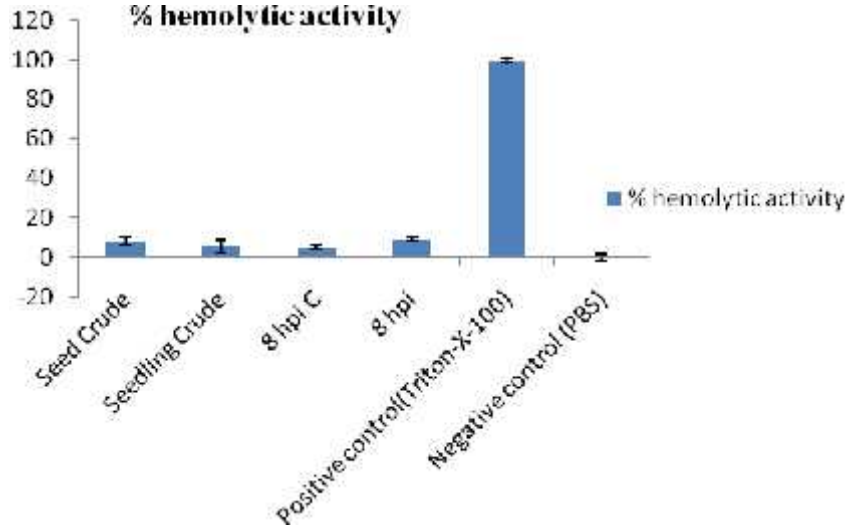

Fig. 2. Hemolytic activity of protein extracts of *P. corylifolia*. Each bar is determinant of mean of three readings. hpi: hours post induction, hpiC: hours post induction control.

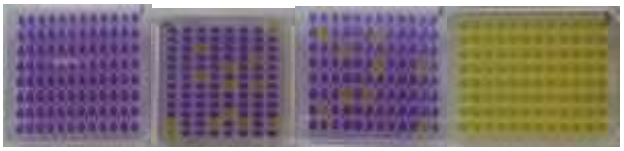

Fig. 3. Mutagenic activity assay (Fluctuation method): Background plate shows natural reversal rate of mutation of test strain (*Salmonella typhimurium*TA98), seedling crude extract is non-mutagenic due to less number of yellow wells as compared to background. A positive control ($K_2Cr_2O_2$) showing all wells yellow is strongly mutagenic.

Table. 1. Fluctuation assay set-up.

Treatments	Constituents (ml)					
	Standards $K_2Cr_2O_2$& H_2N_3	Plant extract	Reagent mixture	S9 mix	Deionized Water	Test strain
Blank	–	–	2.5	–	17.5	-
Background	–	–	2.5	2.0	15.5	0.005
Test sample	–	0.005	2.5	2.0	15.5	0.005
Standard mutagen	0.1	–	2.5	–	17.4	0.005

Table. 2. Mutagenic activity of protein extracts of *Psoralea corylifolia*. Each reading represents mean of three readings. $K_2Cr_2O_2$ and H_2N_3 are positive controls.

Samples	Mutant count for TA98/96	Mutant count for TA100/96	Interpretation
Seed crude	0	8	Non-Mutagenic
Seedling crude	9	8	Non-Mutagenic
8 hpi C	7	5	Non-Mutagenic
8 hpi	7	7	Non-Mutagenic
Background	13	17	-
$K_2Cr_2O_2$	96	94	Mutagenic
H_2N_3	93	91	Mutagenic

Conclusion: In our study potent biological activities of *P. corylifolia* were evaluated under normal and stressed conditions. Significant antioxidant activity was shown by all extracts (crude and protein extracts) estimated by % DPPH radical scavenging activity assay, a maximum of 62% was observed in induced seedling extract. While the extracts possessed low to moderate hemolytic activity and no mutagenic activity was observed in all these extracts. Increase in induced extracts activities as compared to crude extracts might be due to synthesis of some defensive proteins/peptides expressed in response to *fusarium solani* stress could be used in pesticides or fungicide development while non-mutagenic nature of plant assures its safety for human drug development.

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REFERENCES

Ames, B.N., J. McCann, and E. Yamasaki (1975). Methods for detecting carcinogens and mutagens with Salmonella/mammalian-microsome mutagenecity test. Mut. Res. 31(6): 347 – 364.

Arora R., A.S. Dhaker, M. Adhikari, J. Sharma, R. Chawla, D. Gupta, A. Zheleva, Y. Karamalakova, R. Kumar, R.K. Sharma, A. Sharma, S. Sultana, R.K. Sharma, R.P. Tripathi, and V. Gadjeva (2011). Radical scavenging and radiomodulatory effects of *Psoralea corylifolia* Linn. substantiated by in vitro assays and EPR spectroscopy. Z Naturforsch C. 66(1-2): 35-46.

Bhawya, D., and K. R. Anilakumar (2011). Antioxidant, DNA damage protection and antibacterial effect of *psoralea corylifolia*. Asian J. Pharm. Clin. Res. 4 (2): 149-155.

Chopra, B., A.K. Dhingra, and K.L. Dhar (2013). *Psoralea corylifolia* L. (Buguchi) — Folklore to modern evidence: Review. Fitoterapia, 90: 44–56.

Devi, M. K. A., M. Gondi,G. Sakthivelu, P. Giridhar, T. Rajasekaran, and G.A. Ravishankar (2009). Functional attributes of soybean seeds and products, with reference to isoflavone content and antioxidant activity. Food Chem.114(3): 771–776.

Dua, V.K., A. Kumar, A.C. Pandey, and S. Kumar (2013). Insecticidal and genotoxic activity of *Psoralea corylifolia* Linn. (Fabaceae) against *Culex quinquefasciatus* Say, 1823. Parasites & Vectors. 6: 30-37.

Falak, S., and A. Jamil (2013). Expression profiling of bioactive genes from medicinal plant *Nigella sativa*. Appl. Biochem. Biotechnol. 170(6): 1472-1481.

Gidwani, B., R. N. Alsapure, and N. J. Duragkar (2011). Pharmacognostic and standardization and physico-chemical evaluation of *Psoralea corylifolia* linn seeds. Imp J. Pharmacog. Nat. Prod. 1 (1):145–151.

Iqbal, M. J., S. Hanif, Z. Mahmood, F. Anwar, and A. Jamil (2012). Antioxidant and antimicrobial activities of Chowlai (*Amaranthus viridis L.*)

leaf and seed extracts. J. Med. Plants Res. 6(27): 4450-4455.

Jiangning G., W. Xinchu, W. Hou, L. Qinghua, and B. Kaishun (2005). Antioxidants from a Chinese medicinal herb –*Psoralea corylifolia L*. Food Chem. 91(2): 287–292.

Kalita, S., G. Kumar, L. Karthik, and K.V.B. Rao (2011). Phytochemical composition and in vitro haemolytic activity of *Lantana camara L*. (Verbenaceae) leaves. Pharmacology *online* Newsletter. 1: 59-67.

Kiran, B., and K. A. Raveesha (2010). In vitro evaluation of antioxidant potentiality of seeds of *Psoralea corylifolia L*. World ApplSci J. 8 (8): 985-990.

Lee, S. J., K. W. Nam, and W. Mar (2009). Induction of quinone reductase activity by Psoralidin isolated from *Psoralea corylifolia* in Mouse Hepa 1c1c7 cells. Arch. Pharm. Res. 32(7): 1061-1065.

Lewis, D.F., C. Ioannide, and D.V. Parke (1993). Validation of a novel molecular orbital approach (COMPACT) for the prospective safety evaluation of chemicals by comparison with rodent carcinogenicity and *Salmonella* mutagenicity data evaluated by the U.S.NCI/NTP. Mut Res. 291(1): 61-77

Noor, N., R.A. Sarfraz, S. Ali, and M. Shahid (2014). Antitumour and antioxidant potential of some selected Pakistani honeys. Food Chem. 143: 362-366.

Pontiki, E., D. Hadjipavlou-Litina, K. Litinas, and G. Geromichalos (2014). Novel cinnamic acid derivatives as antioxidant and anticancer agents: Design, synthesis and modeling studies. Molecules. 19(7): 9655-9674.

Priya, C.L., G. Kumar, L. Karthik, and K.V.B. Rao (2010). Antioxidant activity of *Achyranthes aspera* Linn stem extracts. Pharmacology online. 2(2): 228-237.

Qiao, C.F., Q.B. Han, J.Z. Song, S.F. Mo, L.D. Kong and H.F. Kung, and H.X. Xua (2006). Quality assessment of fructus psoralea. Chem Pharm Bull. 54(6): 887-890.

Rastegari, E., Z. Ahmad, D.F. Spencer, and M. Ismai (2011). Two-dimensional profiling of proteins from *Curculigo latifolia* fruit by three different extraction protocols. J Med. Plants Res. 5(16): 3719-3724.

Razak, M.F.A., K.E. Aidoo, and A. G.G. Candlish (2007). Mutagenic and cytotoxic properties of three herbal plants from South-east Asia. Tropic Biomed. 24(2): 49–59.

Sah, P., D. Agrawal, and S.P. Garg (2006). Isolation and identification of furocoumarins from the seeds of *Psoralea corylifolia L*. Indian J Pharma Sci. 68(6): 768-771.

Servili, M., B. Sordini, S. Esposto, S. Urbani, G. Veneziani, I.D. Maio, R. Selvaggini, and A. Taticchi (2014). Biological activities of phenolic compounds of extra virgin olive oil. Antioxidants. 3(1): 1-23.

Shahid, M., S.A. Bukhari, Y. Gul, H. Munir, F. Anjum, M. Zuber, T. Jamil, and K.M. Zia (2013). Graft polymerization of guar gum with acryl amide irradiated by microwaves for colonic drug delivery. Int J of Biol Macromol. 62:172-179.

Singariya, P., K.K. Mourya, and P. Kumar (2012). Antimicrobial activity of the crude extracts of *Withania somnifera* and *Cenchrus setigerus* In-vitro. PHcog. J. 4(27): 60-65.

Tadhani, M.B., V.H. Patel, and R. Subhash (2007). In vitro antioxidant activities of *Stevia rebaudiana* leaves and callus. J. Food Composit. Anal. 20(3-4): 323-329.

Tang, S.Y., M. Whiteman, Z.F. Peng, A. Jenner, E.L. Yong, and B. Halliwell (2004). Characterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional chinese medicines. Free Radic. Biol. Med. 36(12):1575-1587.

Xiao, G., G. Li, L. Chen, Z. Zhang, J-J Yin, T. Wu, Z. Cheng, X. Wei, and Z. Wang (2010). Isolation of antioxidants from *Psoralea corylifolia* fruits using high-speed counter-current chromatography guided by thin layer chromatography-antioxidant autographic assay. J Chromat A. 1217(34): 5470–5476.

Zuber, M., S. Tabasum, T. Jamil, M. Shahid, R. Hussain, K. Feras, and K. Bhatti (2014). Biocompatibility and microscopic evaluation of polyurethane–poly (methyl methacrylate)–titanium dioxide based composites for dental applications. J. Appl. Polym. Sci. 131(3), doi: 10.1002/app. 39806.