EXPLORATION OF MEDICINAL PROPERTIES OF *Macrotyloma uniflorum* – AN IMPORTANT GRAIN LEGUME CROP PLANT

Priyanka RAO*, Nikhil MEHTA*, Raman SAINI*

* *Department of Biotechnology, Kurukshetra University, Kurukshetra-136 119, India*
Corresponding author: Raman Saini, Department of Biotechnology, Kurukshetra University, Kurukshetra - 136119, India, Phone: +91-9466218321,email: ramanksaini@rediffmail.com

Abstract. Use of plants in treatment of many diseases has been increased tremendously for enhancement of spectra of plant based natural drugs. So, in this view the present research explore the presence of different phytochemicals, antibacterial, antioxidant and anticancer property of extracts isolated from different parts of *Macrotyloma uniflorum*. Optimized various biophysical parameters for efficient extraction using Soxhlet and evaluated by antibacterial activity. Extracts extracted with 80% methanol at 75˚C for 12 Soxhlet cycles and at their natural pH shows maximum zone of inhibition with 10.1-28.6 mm diameter. MIC ranges from 1.5-4.5 mg/ml against different bacterial strains. Among the different bacterial strains tested, Gram negative bacteria showed higher susceptibility as compare to Gram positive bacteria. Extracts evaluated for the various important phytochemicals found to contained alkaloids, saponins, tannins, flavonoids, steroids and phenols. The antioxidant activity evaluated by different methods such as DPPH, FRAP, DMPD and hydrogen peroxide radical scavenging, among the different extracts seed coat extract shows best antioxidant activity with 73%, 76%, 74% and 70% at the concentration of 100 mg/ml respectively. Among the anticancer activity checked by SRB Assay against human renal cell adenocarcinoma cancer cell line 786-O, the seed coat extract exhibits highest anticancer potential with 66.2% viability of cells. Overall, the results show that the *Macrotyloma uniflorum* have various medicinally important properties and it could be not only used as functional food but also the source of new medicines that may be used to treat various disorders caused due to bacterial infections or oxidative stresses.

Keywords: Antibacterial; Anticancer; Antioxidant; *Macrotyloma uniflorum*, Phytochemicals; Plant extract.

Abbreviations: DPPH: 2,2-diphényl-1-picrylhydrazyl, **DMPD:** N, N-Dimethyl-p-phenylene diamine dihydrochloride, **SRB:** Sulforhodamine B

INTRODUCTION

A large proportion of population is affected by diseases caused by bacterial pathogens and disorders associated with oxidative stress including dangerous cancer. In the recent times, attention has been shifted to plant based natural drugs in place or in addition to synthetic drugs. In this line, antibiotics are of more concerns because of reduced susceptibility of bacterial strains to antibiotics and development of drug resistant bacteria [43]. Besides ineffectiveness, these antibiotics have some side effects as well such as hypersensitivity, immunosuppressant and allergic reaction. Another matter of concern related to human health is oxidative damage that plays a noteworthy pathological role in human diseases like cancer, cardiovascular diseases, hypertension, diabetes, emphysema, cirrhosis, atherosclerosis and arthritis etc. [29]. Among the diseases, cancer is the second leading cause of death worldwide and it was responsible for 8.8 million deaths in 2015 and it is estimated to increase to 11.4 million by 2030 [42]. Over the next two decades, the number of new cases of cancer is expected to rise by about 70%. Therefore, to make a control over it there is an urgent need to hunt safer, low cost and efficient medicinal plants and in particular food plants or their derivatives which are helpful in prevention or treatment of such disorders. It is estimated that today more than 25% of the modern medicines are directly or indirectly derived from plants [9]. Many medicinal plants have been studied for their antimicrobial, antioxidant, anticancer, antidiabetic activity and for the treatment of other diseases and are used by a large population [8]. Most of them are non-cultivated medicinal plants as compare to food crops and in particular grain legume crop plants. Legumes occupy an important place in human diet as they are good source of protein, lipids, dietary fibres, starch, vitamins and other micronutrients. Besides these nutritional compounds legumes are also rich in non-nutrient bioactive compounds [34].

Among the various legume plant species, *Macrotyloma uniflorum* commonly known as Horsegram or Kulthi, a member of fabaceae family is evaluated in the present study using two varieties brown and black seed coat (Figure 1 A). It is having excellent nutritional and remedial properties [3]. Traditionally it is used to cure kidney stones, asthma, bronchitis, leucoderma, urinary discharges, heart diseases, piles etc [3, 30]. It also possesses anti-diabetic [16], anti-ulcer activity [6], anti-hypercholesteremic activity [23] and helps in dietary management of obesity [35] due to presence of useful bioactive compounds. Recently, in one study it was found that horsegram seeds prevent atherosclerosis in rats [36]. Keeping all this in view, the present study was carried out with the aims, to optimize the efficient extraction system for the preparation of extracts using Soxhlet apparatus, phytochemical screening of obtained extracts and to find the antibacterial, antioxidant and anticancer potential of extracts of *Macrotyloma uniflorum* an nutrient rich important grain legume crop.

MATERIALS AND METHODS

Extract preparation

Different parts (stem, root, seed and seed coat) of two varieties (brown and black) of *Macrotyloma uniflorum* were collected in late October from local cultivated field grown plants and then cleaned, dried in shade at room temperature and powdered. While seed coats harvested from one day old water-soaked seeds were shade dried along with the remnant water used for soaking and then powdered for the preparation of extracts. Then various extracts were prepared using powdered material in Soxhlet apparatus by following the various parameters that affects extraction including number of extraction cycles (4-14 cycles), type of solvent (acetone, ethyl acetate, butanol, ethanol, methanol or water), solvent concentration (20-100%) and different extraction temperature (50-100ºC). The so obtained solvent extracts were allowed to evaporate the solvent and remaining powdered (concentrated) form stored at 4°C for further use. Optimal extraction was assessed by using antibacterial assay.

Antibacterial assay

Extracts of different parts of both the varieties of *M. uniflorum* were tested for antibacterial activity by agar well diffusion method against both Gram positive and Gram negative bacteria (*Bacillus subtilis*, *Bacillus cereus, Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa).* Stock solutions of crude plant extracts were prepared in the DMSO at their natural pH with the concentration of 100 mg/ml. Different bacterial strains were grown overnight on Muller Hinton Agar medium. Overnight grown cultures were swabbed uniformly onto the individual plates using sterile cotton swabs and then 5 wells of 6 mm diameter were made on each plate. Different dissolved extracts were poured into each well in equal amount on all plates swabbed with different bacterial strains. The commercial antibacterial drug cefotaxime (5 mg/ml) was used as positive control and dissolving solvent as negative control. The plates were incubated at 37°C for 24 h and after that measured the different zones of inhibition formed around the well. Data taken as average values obtained from experiments performed in triplicate.

Minimum inhibitory concentration (MIC) of seed coat extract of black variety was evaluated by using agar dilution method [26]. The petridishes containing LB medium fortified with different concentrations (0.5-10 mg/ml) of extract were prepared and swabbed with different active bacterial strains individually, by using cotton swab and then incubated at 37ºC for 24 hours. For the MIC against each bacterium, all the plates were visually observed for no visible bacterial growth. The test was performed in triplet with DMSO as negative control.

Antioxidant assays

The antioxidant potential of different extracts prepared form black variety was determined by following the protocols of four different methods including Ferric reducing antioxidant power (FRAP) assay, H_2O_2 scavenging assay, DPPH free radical scavenging activity and DMPD (N, N-Dimethyl-p-phenylene diamine dihydrochloride) method [2, 10, 13, 15], respectively.

Phytochemical screening

Phytochemical screening of seed coat extracts of both the varieties was done to check the presence of different metabolites including alkaloids, glycosides, flavonoids, tannins, saponins, sugars, proteins, steroids and phenols, by following the standard protocols of preliminary qualitative and quantitative phytochemical screening [11, 24, 25].

Anticancer activity

The anticancer activities of extracts of black variety were checked against the Human Renal cell Adenocarcinoma cell line 786-O, at Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai, by using the SRB assay [38]. For the experiment different extracts with concentrations 20, 40, 60 & 80 µg/ml were taken into 96 well microtiter plates containing cells. After 24 h, cell population was measured at the time of drug addition (Tz) by *in-situ* fixing the cells with TCA (trichloro acetic acid). Anticancer drug, Adriamycin (Doxorubicin) was used as a positive control and the experiment was repeated thrice. In this assay, staining was done with Sulforhodomine B solution and the absorbance was measured on an Elisa Plate Reader at a wavelength of 540nm. Percent growth was calculated and expressed as the ratio of average absorbance of test well to that of control wells *100. Percentage growth inhibition was calculated as: [(Ti-Tz)/(C-Tz)] x 100, where Ti is test growth; Tz is growth at time zero; C is control growth. For checking cell viability against drug concentration of tested samples, linear regression method was used.

RESULTS

Extract preparation

Among the different solvents (ethyl acetate, acetone, 1- butanol, ethanol, methanol, and water) used for extraction of bioactive compounds using Soxhlet, no significance difference was observed except water showing least while methanolic extracts of different parts of both the varieties exhibit maximum antibacterial activity (data not shown). Different concentrations of methanol (20-100%) tested, maximum antibacterial activity was found at its eighty percent concentration. Above this concentration there was slight decrease in the activity while below this activity was decreased rapidly (data not shown).

Among the number of Soxhlet extraction cycles, a progressive increase in activity of the extracts with increasing the number of cycles was observed. This increase was up to the 12 cycles afterwards no significant increase found.

For the optimal extraction temperature tested using methanol as solvent, it was found that 75ºC shows maximum antibacterial activity. Above the temperature of 80ºC there was slightly decrease in the activity of the extracts. There was significant difference in the activity below the temperature 65ºC with very less antimicrobial activity of extract at lower temperatures (data not shown).

Antibacterial assay

All the extracts of *M. uniflorum* checked for antibacterial activity were found to possess significant inhibitory activity on all selected strains of bacteria. The inhibition zones of the extracts against the bacterial strains tested were found to be in the range of 10.1-28.6 mm in diameter (Figure 1 B). Among the extracts prepared from different parts and dissolved in

Table 1. Antibacterial activity of methanolic extracts obtained from different parts of the two varieties of *Macrotyloma uniflorum* against different bacterial strains

Bacterial strains	Antibacterial activity of extracts of *M. uniflorum*[1] Average zone of inhibition (values in mm)[2]								
	Variety 1 (Brown)				Variety 2 (Black)				Standard (Cefotaxime)
	Stem	Seeds	Seed coat	Root	Stem	Seeds	Seed coat	Root	
E. coli	19.4	22.3	24.4	13.4	21.3	24.3	26.4	14.5	32.2
P. aeruginosa	20.6	23.6	26.6	13.6	21.6	25.0	28.6	15.2	33.6
B. subtilis	17.9	20.1	23.4	11.9	19.6	22.7	25.4	13.4	29.4
B. cereus	16.8	18.4	21.2	10.8	17.9	21.5	24.3	11.8	28.2
S. aureus	16.0	17.6	20.2	10.1	16.9	20.2	22.6	10.6	27.5

[1] Extracts extracted with 80% methanol at 75˚C for 12 Soxhlet cycles and at their natural pH.
[2] Mean values of experiments repeated thrice.

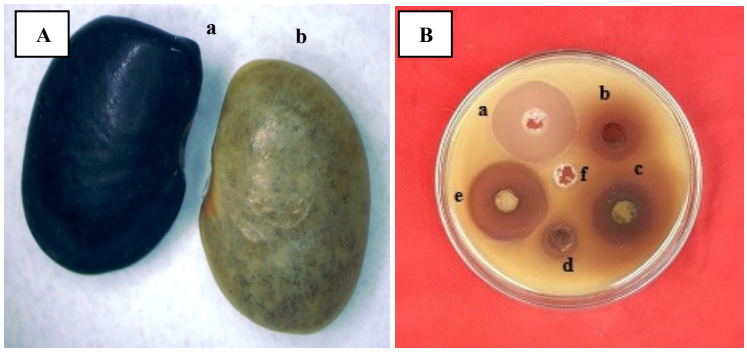

Figure 1. A: Two different varieties of *Macrotyloma uniflorum* - a. Black, b. Brown; **B:** Antibacterial activity of extracts prepared from different parts of the black variety of *Macrotyloma uniflorum* against *Pseudomonas aeruginosa* : **a** - Positive control (cefotaxime), **b** - Stem, **c** - Seeds, **d** – Roots, **e** – Seed coat, **f** - Negative control.

Table 2. Antioxidant activity of different extracts of black variety of *M. uniflorum* at different concentrations using different methods including DPPH activity, Ferric reducing power assay, DMPD scavenging activity and hydrogen peroxide scavenging activity

Method	Concentration (mg/ml)	Different extracts (values in % Inhibition)				Standard
		Seed coat	Seed	Stem	Root	Ascorbic acid
2,2-diphényl-1-picrylhydrazyl (DPPH)	20	52	42	37	30	50
	40	56	47	42	37	58
	60	62	56	48	44	69
	80	68	63	52	47	75
	100	73	68	62	51	80
Ferric reducing power assay (FRAP)	20	56	50	45	35	68
	40	60	57	48	41	72
	60	66	62	55	49	76
	80	72	69	63	52	79
	100	76	72	66	55	83
N, N-Dimethyl-p-phenylene diamine dihydrochloride (DMPD)	20	52	42	38	31	52
	40	61	48	43	39	63
	60	67	57	50	44	74
	80	70	64	54	48	77
	100	74	69	63	52	82
Hydrogen peroxide radical scavenging assay	20	50	40	34	30	50
	40	54	46	42	34	58
	60	57	54	48	42	73
	80	63	58	52	46	78
	100	70	64	60	50	80
[1]TAA %	**100**	**73.25**	**68.25**	**62.75**	**52.00**	**81.25**

[1]Total average antioxidant activity of extracts obtained using different methods at the concentration of 100mg/ml.

DMSO at their natural pH (6.0), seed coat extracts of both the varieties showed the highest antibacterial property against all strains tested, while between the varieties black seed coat variety was found best with maximum zone of inhibition (~29 mm) which was close to the drug cefotaxime (~34 mm), used as a positive control (Table 1). From the results it was also observed that the extracts showed more inhibitory activity against the Gram negative bacteria than the Gram positive bacteria.

Minimum inhibitory concentration (MIC) of seed coat extracts was found in the range between 1.5 - 4.5 mg/ml when tested against different bacteria. The least MIC value of 1.5 mg/ml was obtained against the Gram negative bacteria (*P. aeruginosa*), whereas the highest MIC value of 4.5 mg/ml was observed against *B. cereus*. Other bacteria including *E.coli, S. aureus* and *B. subtilis* showed MIC values 2, 3 and 3 mg/ml respectively. Healthy lawn of growth of microbes was obtained with no sign of any growth inhibition in negative controls indicating supportive culture conditions for their growth.

Antioxidant activity

All the extracts of *M. uniflorum* showed significant antioxidant activity by using different assays including DPPH scavenging assay, hydrogen peroxide scavenging assay, DMPD assay and FRAP assay. Out of different parts, highest activity was found in seed coat extracts followed by seeds, stem and roots, in all the methods used for analysis. Though seeds coat showed a high antioxidant potential but found less than the pure ascorbic acid which was taken as standard. The total antioxidant activity (TAA) percent value of the seeds coat, seeds, stem and roots were found to be 73.25, 68.25, 62.75 and 52% respectively and that of ascorbic acid (standard) was 81.25% at the concentration 100 µg/ml (Table 2).

Phytochemical screening

The results of phytochemical screening tests of seed coat extracts of *M. uniflorum* have shown the presence of alkaloids, saponins, tannins, flavonoids, steroids and phenols in different quantities while glycosides, proteins and sugars were found absent in both the varieties (Table 3). Among the different metabolites alkaloids, phenols and flavonoids were found more abundant in black variety as comared to brown variety.

Table 3. Phytochemical analysis of seed coat extracts of both the varieties of *Macrotyloma uniflorum*

Parameters	Black variety	Brown variety
Alkaloides	++	+
Tannins	++	++
Sugars	-	-
Saponins	+	+
Phenols	+++	++
Glycosides	-	-
Proteins	-	-
Steroides	+	+
Flavonoides	+++	+

Legend: +++ (Much abundant), ++ (less abundant), + (minute), - (absent)

Anticancer activity

The anticancer activity of the extracts was checked against the human renal cell adenocarcinoma cancer cell line 786-O. It was found that the seed coat extract exhibits highest anticancer potential against the tested cell line with 66.2% viability of the cells at the concentration of 80 µg/ml though it was significantly different and very high compared to standard drug (Adriamycin), this difference might be due to crude nature of plant extracts as compare to pure drug (Figure 2).

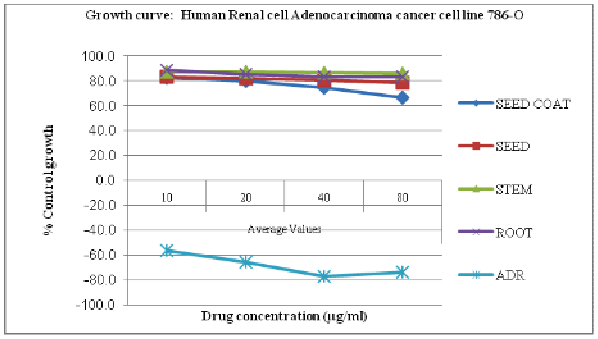

Figure 2. Effect of different extracts of black variety of *M. uniflorum* on growth of Human Renal cell Adenocarcinoma cancer cell line 786-O, checked by using SRB assay

DISCUSSION

The extracts obtained from different parts of the two varieties of the plant *Macrotyloma uniflorum* were screened for antibacterial activity while antioxidant and anticancer activities with extracts of more responsive black variety. Based on antibacterial activity, most significant seed coat extracts were evaluated for phytochemical screening. For efficient evaluation of medicinal potential of a plant extract, it should be first prepared efficiently from the raw plant materials. In this context, various parameters that were optimized includes Soxhlet cycles (12), solvent used during extraction (methanol), concentration of solvent (80%), extraction temperature (75ºC), showed the maximum zones of inhibition against the bacteria tested. The number of Soxhlet cycles affects the quality of the extracts much, with increasing the number of Soxhlet cycles up to 12 number that might be due to more the compounds dissolves in the extraction solvent but after that no increase in the activity of the extracts was noticed which may be because almost all compounds that can be extracted has been dissolved in the solvent and been extracted [26, 40]. Successful extraction of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. In our study, different solvents with polarity ranges from low (ethyl acetate) to high (water) were taken for the extraction of compounds. It was found that extracts obtained using 80% of methanol has high antibacterial property than its pure form and other solvents which are in contradiction to earlier reports [28] showing equal antibacterial effects of extracts isolated in absolute methanol and water

though in our case and many other plant species an hydroalcoholic form at different ratios found better as compare to their pure form [19, 27]. The extraction temperature of the solvent was found to be 75˚C, at which active compounds that are responsible for antibacterial activity may show increased solubility. At higher temperatures from the boiling point of methanol (65ºC) up to 80ºC, no significant effect on the quality of extracts was noted while at temperatures lower than 65ºC poor antibacterial activity observed which may be due to poor solubilization of the compounds in the extracts at lower temperatures. Though slightly decrease in the activity was found as the temperature rises above 80ºC, which may be due to the degradation of the active compounds either by burning effect or reactions among them [26, 40].

Antibacterial potential of *M. uniflorum* has been found to be affected by several ways, one important of them is varietal differences, among them black seed coat variety was found best as compare to brown colored variety. Such varietal differences affecting antibacterial activity has been reported earlier also in different plant species like water chestnut [4], *Rosa indica* [33] and *Labisia pumila* [20] etc. The reason behind such variations may be the differences in qualitative or quantitative or both of different metabolites in black and brown varieties [37] and similarly reported in different varieties of other plant species also such as *Rosa indica* [33] and *Labisia pumila* [20]. Plant part used is also an important factor affecting antibacterial activity as evidenced in several of the plant species like *Cicer arietinum* [18], *Sesbania aculeata* [26]. Among the different parts of *Macrotyloma uniflorum,* the seed coat extracts of black variety showed highest antibacterial activity against all the bacterial strains in comparison to the other parts tested. The present results are supported by earlier reports showing the importance of the seed coats affecting antioxidant potential because of the presence of more phenolics and tannins in seed coat [41] and the compounds like tannins and phenolic are responsible for antibacterial activity [28].

The extracts showed potent activity against both Gram positive and Gram negative bacteria showing the broad spectrum of action though highest antibacterial activity was observed against the *P. aeruginosa* followed by other bacteria tested. The differential activity against different strains may be because of either the presence or concentration of some specific components in the extracts that inhibits the growth of Gram negative bacteria more or because of the difference in some properties of Gram positive and Gram negative bacteria [19]. Similar results are also observed in other legumes such as *Sesbania aculeata* [26] as well as in other plant species [14].

There are several reports showing antibacterial activity of plant extracts of *M. uniflorum* [6, 21, 28] and other plant species [20, 33] but either MIC values have been not studied or studies confined to few parts of the plants only as well as wherever MIC checked in other plant species particularly edible foods mostly found very high values [5, 12]. In this concern, the affectivity of the extracts prepared from different parts was further evaluated based on MIC values against the tested bacteria, which ranges in between 1.5 - 4.5 mg/ml showing that the extracts not only possess antibacterial activity but also acts at low doses, which increases the importance of the crop being utilized as edible grain legume.

In phytochemical screening, extracts of *M. uniflorum* were found to contain many metabolites. In previous studies it has been found that different phytochemicals are present in the seeds [28, 31] and other parts of this crop [39]. For the first time phytochemical screening of the extracts obtained from seed coats alone were done and found the presence of different compounds that were responsible for the medicinal properties of this plant. The results contrast with earlier findings, showing the absence of alkaloids, tannins, flavonoids and phenolic compounds [39] and alkaloids, flavonoids and saponins [28] in the extracts of seeds while our results are in accordance to Ramesh *et al*. (2011) [31], for the presence of metabolites in extracts of seeds except glycosides that was not found in our study and saponins which was not observed by the earlier researchers though we found it in the extracts prepared from seed coats only as compared to earlier reports using whole seeds further the differences in presence of metabolites might be because of varietal differences.

All the extracts tested for their antioxidant potential using different assays showed antioxidant activity. Among the different parts, seed coat extract showed the highest antioxidant activity using FRAP assay in which Fe^{3+} get converted into Fe^{2+} by the reducing power of the compounds present in the extract which indicates the antioxidant potential of the extract. Activity gets increased by increasing the concentration of the extracts, similarly increases in activity has been observed in other plant species also [2, 44]. Although there are some reports showing antioxidant activity of *Macrotyloma uniflorum* seeds and other parts [31, 32, 37] but that are either limited to few methods of evaluation of antioxidant activity or plant part used while in our study different parts of the plant including seed coat has been explored by using four different methods including FRAP, DPPH, DMPD and hydrogen peroxide radicals. Out of the four methods DMPD and hydrogen peroxide radicals as well as seed coats material have not been much studied earlier.

Today, many drugs have been derived from plants to treat cancer, such as vinblastine and vincristine from *Catharanthus roseus* [17], taxol from *Taxus brevifolia* [7] etc. which leads to the screening of more and more therapeutically important plants having phytochemicals exhibiting anticancer potential. In this context, *in vitro* cytotoxicity of *M. uniflorum* extracts has been checked by the SRB assay against human renal cell adenocarcinoma cancer cell line (786-O) and found to have anticancer potential in seed coat extract with

66.2% viability. Earlier, cytotoxicity of whole plant extract of this plant has been also checked against oesteosarcoma cell line MG-63, showing moderate anticancer activity with 82% and 74% viability in methanol and ethanol extracts, respectively which is high as compare to our results [6]. This difference in activity might be due to either different cell line or because of the source of extract i.e. seed coats alone having the highest active metabolites as compare to whole plant extract.

In conclusion, by using the optimized protocol efficiently prepared the extracts from different parts of the two varieties of *Macrotyloma uniflorum* and out of them seeds coat extract of black variety showed highest antibacterial activity, antioxidant property and anticancer potential. Seed coat being the richest source of natural antioxidants and other important metabolites of this plant, present study suggests consuming the seeds along with its coat rather than polished dal or its sprouts to get the maximum medicinal benefits along with nutritional values of this important grain legume plant. In future, further characterization of its metabolites could be exploited to formulate the new drugs or supplements that are safe, cheap and efficient to tackle various ailments to pertain the wellbeing of humans.

Acknowledgement. *In vitro* SRB assay for anti-cancer activity evaluation of extracts was done at Anti-Cancer Drug screening facility (ACDSF) at ACTREC, Tata Memorial Centre, Navi Mumbai. Priyanka Rao is thankful to UGC, New Delhi for the award of Junior Research Fellowship.

REFERENCES

[1] Anderson, J.W., Smith, B.M., Washnock, C.S., (1999): Cardiovascular and renal benefits of dry bean and soybean intake. The American Journal of Clinical Nutrition, 70(3): 464-474.

[2] Benzie, I.F., Strain, J.J., (1999): Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In Methods in Enzymology, 299: 15-27.

[3] Bhartiya, A., Aditya, J.P., Kant, L., (2015): Nutritional and remedial potential of an underutilized food legume horsegram (*Macrotyloma uniflorum*): a review. Journal of Applied Pharmaceutical Science, 25(4): 908-920.

[4] Biswas, K.K., Faruk, M.O., Amin, M. Z., Shaha, R.K., (2014): Antibacterial activity of two varieties of water chestnuts (*Trapa sp.*). Journal of Bio-Science, 20: 115-123.

[5] Bussmann, R.W., Malca-García, G., Glenn, A., Sharon, D., Chait, G., Díaz, D., Aguirre, C., (2010): Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. Journal of Ethnopharmacology, 132(1): 101-108.

[6] Chakraborty, P., Abraham, J., (2016): Antimicrobial and Cytotoxic Effects of *Macrotyloma uniflorum* Extract. International Journal of Pharmacognosy and Phytochemical Research, 8(8): 1334-1340.

[7] Cragg, G.M. (1998): Paclitaxel (Taxol®): A success story with valuable lessons for natural product drug discovery and development. Medicinal Research Reviews, 18(5): 315-331.

[8] Dabur, R., Gupta, A., Mandal, T.K., Singh, D.D., Bajpai, V., Gurav, A.M., Lavekar, G.S., (2007): Antimicrobial activity of some Indian medicinal plants. African Journal of Traditional, Complementary and Alternative Medicines, 4(3): 313-318.

[9] De Silva, T., (1997): Industrial utilization of medicinal plants in developing countries. Medicinal Plants for Forest Conservation and Health Care. FAO, Rome, 34-44.

[10] Da Silva, L.C.N., da Silva Júnior, C.A., de Souza, R.M., Macedo, A.J., da Silva, M.V., dos Santos Correia, M.T., (2011): Comparative analysis of the antioxidant and DNA protection capacities of *Anadenanthera colubrina*, *Libidibia ferrea* and *Pityrocarpa moniliformis* fruits. Food and Chemical Toxicology, 49(9): 2222-2228.

[11] Edeoga, H.O., Okwu, D.E., Mbaebie, B.O., (2005): Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4(7): 685-688.

[12] Ehiowemwenguan, G., Emoghene, A.O., Inetianbor, J.E., (2014): Antibacterial and phytochemical analysis of Banana fruit peel. IOSR Journal of Pharmacy, 4(8): 18-25.

[13] Elansary, H.O., Salem, M.Z., Ashmawy, N.A., Yacout, M.M., (2012): Chemical composition, antibacterial and antioxidant activities of leaves essential oils from *Syzygium cumini* L., *Cupressus sempervirens* L. and *Lantana camara* L. from Egypt. Journal of Agricultural Science, 4(10): 144-152.

[14] Elisha, I.L., Botha, F.S., McGaw, L.J., Eloff, J.N., (2017): The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. BMC Complementary and Alternative Medicine, 17(1): 133.

[15] Fogliano, V., Verde, V., Randazzo, G., Ritieni, A., (1999): Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. Journal of Agricultural and Food Chemistry, 47(3): 1035-1040.

[16] Gupta, L.H., Badole, S.L., Bodhankar, S.L., Sabharwal, S.G., (2011): Antidiabetic potential of α-amylase inhibitor from the seeds of *Macrotyloma uniflorum* in streptozotocin-nicotinamide-induced diabetic mice. Pharmaceutical Biology, 49(2): 182-189.

[17] Guéritte, F., Fahy, J., (2005): The vinca alkaloids. In Anticancer agents from natural products, pp. 131-143. CRC Press.

[18] Kan, A., Özçelik, B., Kartal, M., Özdemir, Z.A., Özgen, S., (2010): *In vitro* antimicrobial activities of *Cicer arietinum* L. (Chickpea). Tropical Journal of Pharmaceutical Research, 9(5): 475-481.

[19] Kang, C.G., Hah, D.S., Kim, C.H., Kim, Y.H., Kim, E., Kim, J.S., (2011): Evaluation of antimicrobial activity of the methanol extracts from 8 traditional medicinal plants. Toxicological Research, 27(1): 31-36.

[20] Karimi, E., Jaafar, H.Z., Ghasemzadeh, A., Ebrahimi, M., (2015): Fatty acid composition, antioxidant and antibacterial properties of the microwave aqueous extract of three varieties of *Labisia pumila* Benth. Biological Research, 48(9): 1-6.

[21] Kawsar, S.M.A., Serajuddin, M., Huq, E., Nahar, N., Ozeki, Y., (2008): Biological investigation of *Macrotyloma uniflorum* extracts against some pathogens. Journal of Biolgical Sciences, 8(6): 1051-1056.

[22] Kumar, D., (2006): Horsegram research: An introduction. Horsegram in India, pp. 1-10.
[23] Kumar, D.S., Prashanthi, G., Avasarala, H., Banji, D., (2013): Antihypercholesterolemic effect of *Macrotyloma uniflorum* (Lam.) Verdc (Fabaceae) extract on high-fat diet-induced hypercholesterolemia in Sprague-Dawley rats.Journal of Dietary Supplements, 10(2): 116-128.
[24] Kumar, R.S., Venkateshwar, C., Samuel, G., Rao, S.G., (2013): Phytochemical screening of some compounds from plant leaf extracts of *Holoptelea integrifolia* (planch.) and *Celestrus emarginata* (Grah.) used by Gondu tribes at Adilabad District, Andhrapradesh, India. International Journal of Engineering Science Invention, 2: 65-70.
[25] Madhu, M., Sailaja, V., Satyadev, T. N. V. S. S., & Satyanarayana, M. V., (2016): Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. Journal of Pharmacognosy and Phytochemistry, *5*(2): 25-29.
[26] Mehta, N., Rao, P., Saini, R., (2018): Efficient extraction of thermostable metabolites of *Sesbania aculeata* and *in vitro* evaluation for their antibacterial potential. Analele Universităţii din Oradea, Fascicula Biologie, 1: 33-41.
[27] Onivogui, G., Letsididi, R., Diaby, M., Wang, L., Song, Y., (2016): Influence of extraction solvents on antioxidant and antimicrobial activities of the pulp and seed of *Anisophyllea laurina* R. Br. ex Sabine fruits. Asian Pacific Journal of Tropical Biomedicine, 6(1): 20-25.
[28] Parvathiraj, P., Sudhakaran, M.R., Athinarayanan, G., Narayanan, K.R., (2015): Phytochemical Analysis and Antibacterial Activity of Seed Extracts of *Macrotyloma Uniflorum* (Horsegram). The Asia Journal of Applied Microbiology, 2(1): 1-9.
[29] Pham-Huy, L.A., He, H., Pham-Huy, C., (2008): Free radicals, antioxidants in disease and health. International Journal of Biomedical Science, 4(2): 89-96.
[30] Prasad, S.K., Singh, M.K., (2015): Horsegram- an underutilized nutraceutical pulse crop: a review. Journal of Food Science and Technology, 52(5): 2489-2499.
[31] Ramesh, C.K., Rehman, A., Prabhakar, B.T., Vijay Avin, B.R., Rao, S.J., (2011): Antioxidant potentials in sprouts vs. seeds of *Vigna radiata* and *Macrotyloma uniflorum*. Journal of Applied Pharmaceutical Science, 1(7): 99-110.
[32] Ravishankar, K., Priya, P.S.V.V., (2012): *In vitro* antioxidant activity of ethanolic seed extracts of *Macrotyloma uniflorum* and *Cucumis melo* for therapeutic potential. International Journal of Research in Pharmacy and Chemistry, 2(2): 442-445.
[33] Sahoo, A.M., Chakraborti, C.K., Nayak, S., Kayal, S., (2011): Correlation between phytochemical screening and *in vitro* antibacterial activity study of *Rosa indica* linn. leaves. International Journal of Research in Ayurveda & Pharmacy, 2(5): 1595-1597.
[34] Sánchez-Chino, X., Jiménez-Martínez, C., Dávila-Ortiz, G., Álvarez-González, I., Madrigal-Bujaidar, E., (2015): Nutrient and nonnutrient components of legumes, and its chemopreventive activity: a review. Nutrition and Cancer, 67(3): 401-410.
[35] Sarojini, R., Malathi, M., Vasanth, S., (2015): Antiobesity and Hypolipidemic activity of *Macrotyloma uniflorum* in Albino rats. International Journal of Science and Research, 5(12): 602-605.
[36] Shobana, G., Sivaelango, G., Kumaravel, P., (2012): Evaluation of antihyperlipidaemic effect of *Macrotyloma uniflorum* seed extract in experimental hyperlipidaemia. Research Journal of Pharmacy and Technology, 5(3): 353-356.
[37] Siddhuraju, P., Maheshu, V., Loganayaki, N., Manian, S., (2008): Antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from processed indigenous legumes, *Macrotyloma uniflorum* (Lam.) Verdc. and *Dolichos lablab* L. Food, 2: 159-167.
[38] Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Boyd, M.R., (1990): New colorimetric cytotoxicity assay for anticancer-drug screening. Journal of the National Cancer Institute, 82(13): 1107-1112.
[39] Suriyamoorthy, P., Subrhamanian, H., Kanagasapabathy, D., (2014): Comparative phytochemical investigation of leaf, stem, flower and seed extracts of *Macrotyloma uniflorum* L. Indo American Journal of Pharmaceutical Research, 4(11): 5415-5420.
[40] Suwari Kotta, H.Z., Buang, Y., (2017): Optimization of Soxhlet extraction and physicochemical analysis of crop oil from seed kernel of Feun Kase (*Thevetia peruviana*). In AIP Conference Proceedings 1911, 020005: 1-6.
[41] Tiwari, A.K., Manasa, K., Kumar, D.A., Zehra, A., (2013): Raw horsegram seeds possess more *in vitro* antihyperglycaemic activities and antioxidant properties than their sprouts. Nutrafoods, 12(2): 47-54.
[42] Torre, L.A., Bray, F., Siegel, R.L., Ferlay, J., Lortet-Tieulent, J., (2015): Global Cancer Statistics, 2012. CA: A Cancer Journal of Clinicians, 65: 87-108.
[43] Ventola, C.L., (2015): The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and Therapeutics, 40(4): 277-283.
[44] Vijayalakshmi, M., Ruckmani, K., (2016): Ferric reducing anti-oxidant power assay in plant extract. Bangladesh Journal of Pharmacology, 11(3): 570-572.

Received: 16 November 2018
Accepted: 28 February 2019
Published Online: 4 March 2019
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House