Phytochemical analysis and in vitro antioxidant stress properties of methanol acetate and ethyl extracts of \textit{Tirmania Nivea} and \textit{Tirmania Pinoyi}

Houda Attjioui\textsuperscript{1}, Hamadoun Abba Touré\textsuperscript{2}, Amine Cheikh\textsuperscript{3}, Hafid Mefetah\textsuperscript{4}, Mustapha Draoui\textsuperscript{5}, Mustapha Bouatia\textsuperscript{5}

\textsuperscript{1}Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco
\textsuperscript{2}Team of formulation and quality Control of health Products, Laboratory of Analytical Chemistry, Faculty of Pharmacy, Sciences, Techniques and Technologies University, Bamako, Mali
\textsuperscript{3}Faculty of Medicine and Pharmacy, Abulcasis University, Rabat, Morocco
\textsuperscript{4}Faculty of Medicine and Pharmacy, Paediatrics hospital, Rabat, Morocco
\textsuperscript{5}Team of formulation and quality Control of health Products, Laboratory of Analytical Chemistry, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco

\section*{Article History:}
Received on: 14 May 2020
Revised on: 15 Jun 2020
Accepted on: 17 Jun 2020

\section*{Keywords:}
Tirmania Nivea, Tirmania Pinoyi, Methanol extracts, Ethyl acetate extracts, Oxidative stress

\section*{ABSTRACT}
For thousands of years, truffles have been used as essential foods in different cultures around the world because of their rich nutritional value and their pleasant and characteristic smell. We have studied the effect of truffles (\textit{Tirmania Nivea} and \textit{Tirmania Pinoyi}) extracts on the antioxidant stress properties issued from the Moroccan desert. Antioxidant and anti-free radical activities were studied using three analytical methods: trapping capacity of 1,1-diphenyl-2-picrylhydrazyl, phosphomolybdate, and reducing ferric antioxidant capacity; in addition, phenol and flavonoid levels were measured. The results of the FRAP, DPPH and PPM tests of T. Nivea were respectively $4.112 \pm 0.217$, $0.142 \pm 0.006$, $2.235 \pm 0.110$ mg/mL for methanols and $3.404 \pm 0.096$, $0.080 \pm 0.003$, $0.693 \pm 0.057$ mg/mL for ethyl acetate extracts. The results of the tests of \textit{T. pinoyi} were respectively $3.670 \pm 0.572$, $0.102 \pm 0.004$, $0.907 \pm 0.014$ mg/mL for methanols and $3.404 \pm 0.096$, $0.080 \pm 0.003$, $0.693 \pm 0.057$ mg/mL for ethyl acetate extracts.

For this work, we propose a valorization of the Moroccan truffle in the prevention of oxidative stress.

\section*{INTRODUCTION}
Since the beginning of civilization, desert macrofungi (truffles) have been exploited for food and medicine. The truffle belongs to the genera \textit{Tirmania} and Terfezia of the Terfeziaceae family, order Pezizales, edible fungi, mainly endemic to the semi-arid and arid areas of North Africa and the Mediterranean region (Bouatia \textit{et al.}, 2018).

Macrolfungi have been classified as food and medicines only for the royal family. No citizen has been allowed to benefit from it. During Greek and Roman rule, these mushrooms were imported from Libya and sold in southern Europe (Enshasy \textit{et al.}, 2013).

The collection of truffles is carried out by competent and experienced specialists for this type of flora. Sometimes, animals such as dogs and pigs are used to facilitate the discovery of underground fungi. Truffles are very rich in volatile compounds.
which explains the choice of this exploration technique. For a long time, truffles are consumed raw or cooked as precious food. They have also been used for the benefit of traditional medicine. This use was due to its high fibre, fatty acids, proteins, vitamins, amino acids, minerals, terpenoids, sterols, carbohydrates, and aromatic compounds (Bokhary and Parvez, 1993; Kalač, 2009). Indeed, the natural properties attributed to truffles and their derivatives have served as a field of exploration for scientists to study their added value (Hamza et al., 2016).

The metabolic mechanism in humans is capable of producing free radicals responsible for the oxidation of biomolecules such as lipids, proteins, DNA which can cause cell-death and tissue-damage. (Dubost et al., 2007). It has been observed that oxidizing enzymes, as well as compounds such as ascorbic acid, tocopherols, and phenols, have a protective effect against damage caused by free radicals formed. Regular consumption of natural antioxidants can prevent and correct the imbalance of the antioxidant system caused by accelerated aging, responsible for the development of certain diseases such as cardiovascular disease, cancer, diabetes, and other age-related diseases (Kris-Etherton et al., 2002). Tirmania is a significant source of food for the North African population; it is found in the deserts of Morocco, where it grows spontaneously. In this study, we have sought to further our knowledge of the antioxidant activities of Tirmania in the arid areas of Morocco. Phytochemical screening and antioxidant properties were studied for methanol and ethyl acetate extract.

MATERIALS AND METHODS

Extraction of Tirmania phytochemical compounds

Four grams of Tirmania powder were weighed and soaked separately in 15 mL of an organic solvent such as methanol and ethyl acetate. The mixture was maintained under magnetic agitation or 24 hours at room temperature for 24 hours. The soaked powder was filtered, and the raw extracts were stored at low heat for analyzing.

Phytochemical characterization

Qualitative tests for the characterization of chemical groups (secondary metabolites) were performed using extracts of methanol and ethyl acetate from T. Nivea and T. Pinoyi using the standard procedures defined above.

Saponins test

1 gram of the raw extract was homogenized in 3 mL of distilled water for 15 seconds and stored at ambient temperature for 15 minutes. The presence and persistence of moss (more than 1 cm high) indicate the presence of saponins (Angone et al., 2013).

Anthraquinones analysis

1 gram of extract was mixed with 2 mL of chloroform. The supernatant was collected, and 10% aqueous KOH (v/v) was added to the test tube. After stirring, the red colouration indicated the existence of anthraquinones (Angone et al., 2013).

Polyphenols analysis

The reaction with ferric chloride (FeCl3) was used to reveal the presence of polyphenols. 2 mL of methanol and ethyl acetate extracts were prepared and mixed with 1 or 2 drops of ferric chloride (solubilized in 2% ethanol). The dark blue or green colour indicates the existence of polyphenols (Angone et al., 2013).

Tannins analysis

5 grams of extract per material was homogenized with 10 mL of 80% methanol, stirred for 15 minutes, and the soluble fraction was transferred into test tubes. The presence of tannins was tested by adding FeCl3 (1% in water). The dark blue or green colour indicated the presence of Gallic tannins, while the brown-green colouration indicated the existence of catechetal tannins (Angone et al., 2013).

Flavonoids analysis

Alkaline reagent test: truffle extracts treated with 2 or 3 drops of sodium hydroxide in solution. Formation of an intense yellow colour, which becomes colourless by adding a few drops of sulphuric acid, indicating the presence of flavonoids (Yadav et al., 2017).

Anthracenosids analysis

The ether extract (4mL) was concentrated to 2mL and stirred with 2 mL of 25% ammonia solution. The appearance of a cherry red solution on the top layer indicates the presence of models (anthracenoside aglycones) in the oxidized form (Nabatanzi et al., 2015).

Sterols analysis

An extract was prepared using 1 gram of powdered sample and 20 mL of macerating ether for 24 hours in a water bath. The extract obtained was used to detect sterols.

10 mL of ether macerate was dry evaporated. 1 mL of chloroform was added to the residue. The solution obtained was divided into two assay pieces. One to 2 mL of concentrated sulfuric acid was placed in the bottom of one of the assay tubes, the other serving as a control. The formation of a reddish-brown
Table 1: Qualitative phytochemical screening of *Tirmania Nivea* and *Tirmania Pinoyi*

| Tests/Species          | *Tirmania Pinoyi* | *Tirmania Nivea* |
|------------------------|-------------------|------------------|
| Polyphenols            | +++               | +                |
| Flavonoids             | +++               | +                |
| Tannins                | +++               | ++               |
| Anthracenosids         | +                 | +                |
| Alkaloids              | +++               | -                |
| Anthraquinons          | +++               | ++               |
| Saponosids             | +++               | +++              |
| Sterols                | +                 | +                |

Note: (+++), (++), and (+) signs indicate high presence of active ingredient, medium, and low active ingredient respectively. (-) the sign indicates the absence of an active ingredient.

Table 2: Total phenolic content and total flavonoid content of *T. Pinoyi* and *T. Nivea*

| Truffles extracts      | TPC (mg GAE/100 g of DM) | TFC (mg QE/100 g of DM) |
|------------------------|--------------------------|-------------------------|
|                        | (Mean±SD)                | (Mean±SD)               |
| tn methanol extract    | 9.90±1.50                | 1.60±0.40               |
| tp methanol extract    | 30.50±24.8               | 0.20±0.10               |
| tn Acetate extract     | 47.00±0.60               | 0.40±0.10               |
| tp Acetate extract     | 13.00±0.80               | 0.30±0.10               |

Values are considered significant at p < 0.05

TPC: Total phenolic content; TFC: Total flavonoid content
tn: *Tirmania Nivea*; tp: *Tirmania Pinoyi*

Table 3: Antioxidant and antiradical properties of *Tirmania Pinoyi* and *Tirmania Nivea* extracts

| Matrix/Tests             | Test Phosphomolybdate IC$_{50}$ (mg/mL) | Test FRAP IC$_{50}$ (mg/mL) | Test DPPH IC$_{50}$ (mg/mL) |
|-------------------------|----------------------------------------|-------------------------------|-----------------------------|
| tn methanol extract     | 2.235±0.110                            | 4.112±0.217                  | 0.142±0.006                 |
| tp methanol extract     | 0.907±0.014                            | 3.670±0.572                  | 0.102±0.004                 |
| tn Acetate extract      | 0.858±0.010                            | 3.424±0.034                  | 0.137±0.025                 |
| tp Acetate extract      | 0.693±0.057                            | 3.404±0.096                  | 0.080±0.003                 |
| Ascorbic acid standard  | 0.100±0.001                            | 0.338±0.043                  | 0.020±0.001                 |

Values are considered significant at p < 0.05
tn: *Tirmania Nivea*; tp: *Tirmania Pinoyi*

or purple ring where the two phases meet indicates the presence of sterols (Nabatanzi *et al.*, 2015).

**Alkaloids analysis**

The Truffle extract was homogenized with 1% v/v HCl, heated and filtered. This filtrate was used for the subsequent analysis.

**Mayer’s analysis**

The filtrate was treated with the Mayer reagent. Yellow colour precipitation is considered an indication of the existence of alkaloids (Yadav *et al.*, 2017).

**Determination of total phenolic content (TPC)**

TPC was determined using the Folin-Ciocalteu method (Ghadage *et al.*, 2017; Wolfe *et al.*, 2003). A spectrophotometer measured the absorbance of the developed blue colour at 760 nm. The results were expressed in milligrams of gallic acid equivalent per gram of extract.

**Determination of Total Flavonoid Content (TFC)**

The aluminium chloride colourimetric method was used to quantify the total flavonoid Content (Chougui *et al.*, 2013). The optical density of the extracts and standard solutions was read at 430 nm. The concentration of flavonoids was expressed as milligram equivalents of quercetin per gram of extract.
Antioxidant capacity

DPPH radical scavenging assay

Antioxidant activity was evaluated using a solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in 100 ml of refrigerated methanol (Ghadage et al., 2017; Brand-Williams et al., 1995). 750 μL of truffle extract was introduced into test tubes with 1.75 mL of DPPH solution. The mixture was vortexed and kept in the dark for 30 min. A spectrophotometer measured the absorbance at 517 nm.

Ferric reducing antioxidant power (FRAP) assay

For the different extracts of T. Nivea and T. Pinoyi, the test was performed using the method described by Chougui et al. (2013). After 30 minutes of incubation in the dark at room temperature, the absorbance was measured with a spectrophotometer at 700 nm (Gordon, 1990). The increase in absorbance of the reaction mixture shows a high reducing power.

Phosphomolydate (PPM) assay

The phosphomolydate method using ascorbic acid as the standard is used to determine antioxidant capacity (Odunola et al., 2015). In a test tube, the 500 μl volume of the extract solution is added to 2.5 ml of the reagent (sulphuric acid 0.6 M, sodium phosphate 28 mM, and ammonium molybdate four mM). The tubes were closed and incubated in a water bath at 95°C for 90 minutes. The absorbance was measured by UV spectrophotometer at 695 nm relative to the blank, after cooling the samples to room temperature. The results were expressed in milligram equivalent of ascorbic acid per 100 grams of extract.

Statistical Analysis

Data are reported as mean ± standard deviation. Analyses were performed in triplicate. A unidirectional ANOVA test was performed to analyze the significance of the difference between the different extracts studied. The test is significant if p < 0.05. The IC50 was determined graphically by a linear regression method.

RESULTS AND DISCUSSION

Phytochemical characterization

Phytochemical screening of the truffles revealed some differences in the constituents of the two truffles tested Table 1.

T. Pinoyi was positive for all phytochemicals tested; T. Nivea showed the absence of alkaloids. All truffles had antioxidant activity. The presence of polyphenols in all truffles is probably responsible for the free radical scavenging effects observed.

Determination of total phenolic and flavonoid contents (TPC) and (TFC)

Phenolic compounds are the main secondary metabolites of truffles. They have several pharmacological activities useful in the prevention of chronic diseases. In addition to their antioxidant activities, they can act on free radicals by reducing their oxidative power. The results obtained for the phenol and flavonoid levels of T. Nivea and T. Pinoyi are presented in Table 2.

Phenolic compounds are potent antioxidants; they exert their activity by capturing or trapping free radicals such as pyroxylated and hydroxylated radicals or peroxynitrite and superoxide anions (Carocho and Ferreira, 2013). Phenolic compounds derived from truffles are classified into several families, the main one being flavonoids. The latter have more marked antioxidant or anti-free radical activities than the others (Almeida et al., 2012). In addition to other antioxidants, flavonoids have been more effective in their mechanism of action by trapping oxidants or free radicals (Bravo, 1998). These results are similar to the results in the literature for other desert truffle extracts (Al-Laith, 2010) From our findings on the inhibitory concentration, 50 of soluble extracts of T. Nivea and T. Pinoyi. We can suggest that phenolic compounds may be the main actors of antioxidant or anti-radical activity. The concentration of total phenol was much superior in T. Nivea ethyl acetate extract than in T. Pinoyi ethyl acetate and methanol extracts. T. Nivea has relatively high contents of flavonoids, phytochemicals compounds that are responsible for antioxidant activity and have been primarily reviewed for their health benefits. Phenolic compounds in general and flavonoids, in particular, can contribute to the inhibition of the pro-oxidant effects of proteins, DNA, and lipids. (Yeddes et al., 2013).

Antioxidant capacity

Oxygen is essential in the physiology of life. Also, it can be a source of increased cell damage due to oxidative damage (Elmouttaleb et al., 2012). Oxidative stress is triggered when there is a disorder between the reactive species of oxygen produced and the antioxidant capacity of the cell concerned (Ahmad et al., 2009).

Several in vitro tests were performed to determine antioxidant properties to obtain active substance content (Almeida et al., 2012). Many chronic diseases are due to the action of free radicals. Antioxidants or anti-free radicals fight against these free radicals and protect organisms against various dis-
eases. The mechanism of action is the trapping of reactive oxygen species or the strengthening of antioxidant defence mechanisms (Umamaheswari and Chatterjee, 2008; Toure et al., 2015).

The antioxidant activity, estimated by three different analyses, showed moderate reducing power of methanol and ethyl acetate extracts Table 3 for T. Pinoyi and T. Nivea (IC50 = 4.112±0.217 and 3.670±0.572 mg/mL; 3.424±0.034 and 3.404±0.096 mg/mL respectively), elimination of DPPH radicals (IC50 = 0.142±0.006 and 0.102±0.004 mg/mL; 0.137±0.025 and 0.080±0.003 mg/mL respectively), phosphomolybdenum inhibition (IC50 = 2.235±0.110 and 0.907±0.014 mg/mL; 0.858±0.010 and 0.693±0.057 mg/mL).

Depending on the effect of extracts of T. Nivea and T. Pinoyi on the colour intensity of the diphenyl-1,2-diphenyl-1-picrylhydrazyl solution, their anti-free radical capacity is measured (Almeida et al., 2012). The assay method is used by trapping the DPPH radical by adding another free radical or an antioxidant. The action of the latter causes a discolouration of the initial solution. The low intensity of the colour is relative to the strength of the antioxidant substance or its concentration. The strong anti-radical activity of the tested compound is demonstrated by a significant decrease in the absorbance of the reaction solution. DPPH is a stable radical that produces a violet staining solution in methanol, at room temperature. The DPPH solution gives a high absorbance in the visible spectrum at 515 nm (Saeed et al., 2012; Krishnaiah et al., 2011). In this work, the samples (T. Nivea and T. Pinoyi) tested with methanol extracts, and ethyl acetate showed a low inhibition concentration. According to these results, we notice that truffle extracts may contain phytochemical components capable of trapping potential damage by giving hydrogen to a free radical.

They were using the method based on the formation of phosphomolybdenum compounds by the reduction of Molybdenum (VI) to Molybdenum (V) by extracts. The antioxidant capacity of the different extracts is determined by UV-Visible spectrophotometry with maximum absorption at 765 nm. The results showed that T. Nivea and T. Pinoyi had a strong antioxidant capacity for phosphomolybdenum reduction. Recently, it has been shown in numerous studies that flavonoids and related polyphenols are significant, responsible for the phosphomolybdate scavenging capacity of medicinal plants. (Sharififar et al., 2009; Khan et al., 2012).

The reducing power of the different extracts and the reduction in intensity are responsible for the test solution changing from a yellow to a green colour. The reduction of the ferric complex to ferrous form is due to the presence of reducers or chelators in the solution. The ferrous form can be measured by spectrophotometer at 700 nm. It has been shown in the literature that anti-radical properties are possible by adding a hydrogen atom that is capable of breaking the chain of free radicals (Gordon, 1990; Ramaswamy et al., 2015). The reducing power of the extracts depended on the concentration. At 700 nm, there is a proportionality between absorbance and reducing concentration. The antioxidants present in T. Nivea and T. Pinoyi extracts have caused the reduction of the ferric complex into a ferrous complex. This mechanism has demonstrated the presence of antioxidant compounds.

CONCLUSION

The truffle extract Tirmania Nivea and Tirmania Pinoyi from the desert of Morocco has shown, according to three methods of analysis, antioxidant and anti-radical activities to varying degrees. This work is an update on the nutraceutical potential and antioxidant compounds of T. Nivea and T. Pinoyi, an edible wild desert truffle. It is interesting to note that Burmese truffles appear to be an essential source of several natural antioxidants. Besides, these truffles appreciated by consumers could be used as high antioxidant foods to alleviate oxidative stress responsible for certain chronic diseases. Bioactive compounds from truffles may be a promising alternative source that could be used as important therapeutic agents in the pharmaceutical industry against many diseases.

ACKNOWLEDGEMENT

The authors are grateful to the Team of Analytical’s Chemistry Laboratory for providing laboratory facility for this research work.

Funding Support

There is no specific funding for the preparation of this manuscript.

Conflict of Interest

The authors declares that they have no competing interest.

REFERENCES

Ahmad, I. M., Abdalla, M. Y., Mustafa, N. H., Qnais, E. Y., Abdulla, F. A. 2009. Datura aqueous leaf extract enhances cytotoxicity via metabolic oxidative stress on different human cancer cells. Jordan Journal of Biological Sciences, 2(1):9–14.
Al-Laith, A. A. A. 2010. Antioxidant components and antioxidant/antiradical activities of desert truffle (Tirmania nivea) from various Middle Eastern origins. *Journal of Food Composition and Analysis*, 23(1):15–22.

Almeida, J. R. G. D. S., da Cruz Araújo, E. C., de Araújo Ribeiro, L. A., de Lima, J. T., Nunes, X. P., Lúcio, A. S. C., de Fátima Agra, M., Filho, J. M. B. 2012. Antinociceptive Activity of Ethanolic Extract from Duguetia chrysocarpa Maas (Annonaceae). In *The Scientific World Journal*, volume 2012, pages 1–6, New York. Hindawi Limited.

Angone, S., Mewono, L., Mounanga, M., Medzegue, S., Mendene, H. F. E., Ndong, J. G. M., Siawaya, J. F. D., Souza, A. 2013. Phytochemical screening and cytotoxicity studies of Chrysophyllum pruniforame Pierre ex Engl. barks. *Pharmacognosy Research*, 5(3):195–195.

Bokhary, H. A., Parvez, S. 1993. Chemical Composition of Desert Truffles Terfezia claveryi. *Journal of Food Composition and Analysis*, 6(3):285–293.

Bouatia, M., Touré, H. A., Cheikh, A., Eljaoudi, R., Rahali, Y., Idriissi, O. M. B., Draoui, M. 2018. Analysis of nutrient and antioxidant content of the truffle (Tirmania pinoyi) from Morocco. *International Food Research Journal*, 25(1):174–178.

Brand-Williams, W., Cuvelier, M. E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity.

Bravo, L. 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition reviews*, 56(11):317–333.

Carocho, M., Ferreira, I. C. 2013. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, 51:15–25.

Chougui, N., Tamendjari, A., Hamidj, W., Hallal, S., Barras, A., Richard, T., Larbat, R. 2013. Oil composition and characterisation of phenolic compounds of Opuntia ficus-indica seeds. *Food Chemistry*, 139(1-4):796–803.

Dubost, N., Ou, B., Beeelman, R. 2007. Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chemistry*, 105(2):727–735.

Elmouttaleb, A., Mostafa, A. T., S. U. E. 2012. Effect of tomato and guava juices on oxidative stress in rats after strenuous exercise. *Jordan Journal of Biological Sciences*, 147(619):1–7.

Enshasy, H. E., Elsayed, E. A., Aziz, R., Wadaan, M. A. 2013. Mushrooms and Truffles: Historical Biofactories for Complementary Medicine in Africa and in the Middle East. *Evidence-Based Complementary and Alternative Medicine*, 2013:1–10.

Ghadge, D. M., Kshirsagar, P. R., Pai, S. R., Chavan, J. J. 2017. Extraction efficiency, phytochemical profiles and antioxidative properties of different parts of Saptarangi (Salacia chinensis L.) – An important underutilized plant. *Biochemistry and Biophysics Reports*, 12:79–90.

Gordon, M. H. 1990. The Mechanism of Antioxidant Action in Vitro. *Food Antioxidants*, pages 1–18.

Hamza, A., Zouari, N., Zouari, S., Jdir, H., Zaidi, S., Gtari, M., Neffati, M. 2016. Nutraceutical potential, antioxidant and antibacterial activities of Terfezia boudieri Chatin, a wild edible desert truffle from Tunisia arid zone. *Arabian Journal of Chemistry*, 9(3):383–389.

Kalač, P. 2009. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, 113(1):9–16.

Khan, R. A., Khan, M. R., Sahreen, S., Ahmed, M. 2012. Assessment of flavonoids contents and in vitro antioxidant activity of Launaea procumbens. *Chemistry Central Journal*, 6(1):43–43.

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., Griel, A. E., Etherton, T. D. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine*, 113(9):71–88.

Krishnaiah, D., Sarbatly, R., Nithyanandam, R. 2011. A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing*, 89(3):217–233.

Nabatanzi, A., Kabasa, J. D., Nakalembe, I. 2015. Phytochemicals and Antioxidant Properties of Five Wild Edible Plants Consumed by Pregnant Women in Buikwe District. *Uganda. International J. of Pharmacognosy and Phytochemical Research*, 7(6):1267–1271.

Ondonula, O., Olugbami, J., Gbadegesin, M. 2015. In vitro free radical scavenging and antioxidant properties of ethanol extract of Terminalia glaucescens. *Pharmacognosy Research*, 7(1):49–49.

Ramawswamy, N., Mahitha, B., Archana, P., Ebrahimzadeh, M. H., Srikanth, K., Rajinikanth, M. 2015. In vitro antioxidant and pharmacognostic studies of leaf extracts of cajanus cajan (L.) millsp. *Evidence-Based Complementary and Alternative Medicine*, 12(1):1174–1174.
Sharififar, F., Dehghn-Nudeh, G., Mirtajaldini, M. 2009. Major flavonoids with antioxidant activity from Teucrium polium L. *Food Chemistry*, 112(4):885–888.

Toure, H., Bouatia, M., Idrissi, M. O., Draoui, M. 2015. Phytochemical screening and antioxidant activity of aqueous-ethanolic extracts of Opuntia ficus indica. *Journal of Chemical and Pharmaceutical Research*, 7(7):409–415.

Umamaheswari, M., Chatterjee, T. K. 2008. In vitro antioxidant activities of the fractions of Coccinia grandis L. leaf extract. *African Journal of Traditional, Complementary and Alternative Medicines*, 5(1):61–73.

Wolfe, K., Wu, X., Liu, R. H. 2003. Antioxidant Activity of Apple Peels. *Journal of Agricultural and Food Chemistry*, 51(3):609–614.

Yadav, R., Khare, R. K., Singhal, A. 2017. Qualitative Phytochemical Screening of Some Selected Medicinal Plants of Shivpuri District (M.P.). *International Journal of Life-Sciences Scientific Research*, 3(1):844–847.

Yeddes, N., Chérif, J., Guyot, S., Sotin, H., Ayadi, M. 2013. Comparative Study of Antioxidant Power, Polyphenols, Flavonoids and Betacyanins of the Peel and Pulp of Three Tunisian Opuntia Forms. *Antioxidants*, 2(2):37–51.