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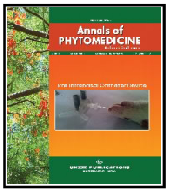

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Formulation, phytochemical and antioxidant activity evaluation of selected Indian medicinal plants

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Aconitum heterophyllum Wall. ex Royale

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Antioxidant activity

FT-IR

Atomic absorption spectroscopy

Abstract

The objective of this research was to develop a polyherbal formulation (ABA PHF) using three different herbs and to evaluate their phytochemicals, physical constants and determination of their antioxidant activity by DPPH method. The PHF authenticated herbs were characterized by studying their morphological and phytochemical analysis. Preliminary screening showed the presence of alkaloids, glycosides, carbohydrates, amino acids and flavonoids in the combination extract. Physical parameters such as loss on drying (LOD), ash values and extractive values have been studied. The antioxidant activity of the combination of extract (100 mg each) was determined using DPPH free radical scavenging method.The results showed that the combination extract has best antioxidant effect at a dose of 300 µg/ml when it was compared with ascorbic acid as the reference standard. All the extracts and PHF showed a dose dependent activity . The result showed that the PHF has more potent antioxidant activity when compared with individual extract. It also shows that the combination of three different herbs in a single formulation is completely compatible with each other which is determined from FTIR. The presence of heavy metal and minerals in the prepared extracts and PHF where analysed using atomic absorption spectroscopy. The results showed that the tested minerals and metals were within the specified limit. The result showed that the PHF has more potent antioxidant activity when compared with individual extract.

1. Introduction

Nowadays, folklore medicine is being re-evaluated by extensive research on different plant species and their therapeutic principles. *Aconitum heterophyllum* Wall. ex Royale (whole plant) (Deepika *et al*., 2014) belongs to the family, Ranunculaceae, a traditional medicinal plant species of India; *Benincasa hispida* (Thumb.) Cogn. (fruit) (Waidyarathna and Ediriweera, 2020) belongs to the family, Cucurbetaceae, a traditional medicinal plant species of India, South east Asia and Japan, *Aegle marmelos* (L.) Corr. (leaves) (Chamila *et al*., 2020) belongs to the family, Rutaceae, a traditional medicinal plant species of India, South east Asia is being prescribed to treat dysentery, dyspepsia, malabsorption, neurological diseases, edema, vomiting, rheumatism, *etc*.

Antioxidant is defined as a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals (Satyendra *et al*., 2012). In turn, these radicals can start chain reactions that damage cells. Antioxidant terminates these chain reactions by removing free radical and inhibits other oxidation reactions. They do so by being oxidized themselves; so, antioxidants act often as reducing agents such as thiols, ascorbic acid, or polyphenols. Many studies had revealed that phenolic content in plants could be correlated to their antioxidant activities. Plants contained phenolic and polyphenol compounds, can act as an antioxidant. Oxidative-process is the most common route for producing free radicals in food, drugs and even in living systems. The majority of free radicals that damage biological systems are oxygen radicals. Antioxidants also act as radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors and metal chelating agents. Due to the effect on immune system, there is a need for natural antioxidants (safe and nontoxic) as compared to synthetic antioxidants (toxic for human).The objective of this work was to assess the antioxidant activity of the combination of extract of polyherbal formulation (ABA PHF) (Muhammad, *et al*., 2016) by *in vitro* studies.

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2. Materials and Methods

2.1 Collection, identification and authentication

The whole plant of *Aconitum heterophyllum* Wall. ex Royale, fruit *of Benincasa hispida* (Thumb.) Cogn. and leaves of *Aeglemar melos* (L.) Corr. were collected from Sri Venkateshwara University, Tirupati, Andhra Pradesh, India.

All these three plants were authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupati - 517502, Andhra Pradesh, India (Voucher No: 0215, 0449, 0389).

2.2 Methods of extraction

2.2.1 Preparation of ethanolic extract

100 g of *A. heterophyllum*, *B. hispida* and *A. marmelos* (L.) were was washed, dried and made into powder form separately through

10 mesh sieve. The separated plant powder was then packed into different Soxhlet apparatus and subjected to hot continuous percolation using ethanol (95%v/v) (hydroalcoholic extract-70:30) as solvent. The extracted solution received from ethanolic extractions was filtered hot through muslin cloth. The filtrate was to be concentrated in a vacuum evaporator at 60^0C and dried in an oven set at 40^0C separately. The dried flakes were pulverised to 80-100 mesh and packed under hygienic condition. Finally, the percentage yield of the dried extract calculated.

2.3 Preliminary phytochemical screening

All the three plants extracts and polyherbal formulation were subjected to qualitative tests for the identification of various active constituents (Singh *et al*., 2015), *viz.*, carbohydrate, glycosides, alkaloids, amino acids, flavonoids, fixed oil, tannins, gum and mucilage, phytosterols, *etc.*, according to standard procedure**.**

2.4 Physicochemical evaluation

All extracts and polyherbal formulation were subjected to physicochemical parameters (Balalakshitha and Kolanjinathan, 2021) such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value were calculated as per Indian Pharmacopoeia.

2.5 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) uses the mathematical process (Fourier transform) to translate the raw data (interferogram) into the actual spectrum. FTIR method is used to obtain the infrared spectrum of transmission or absorption of a fuel sample. FTIR identifies the presence of organic and inorganic compounds in the sample. Depending on the infrared absorption frequency range 600-4000 cm^{-1}, the specific molecular groups prevailing in the sample will be determined through spectrum data in the automated software of spectroscopy (Vanshika *et al*., 2022). In this chapter, FTIR has been used as a novel approach to characterize the variability in fuel stability of various biodiesel/ antioxidant samples.

2.6 Atomic absorption spectroscopy

2.6.1 Heavy metal analysis

Heavy metal analysis of the plant material was performed by preparing acid digestion by consecutive treatment with nitric and sulphuric acid which was further treated with ammonium oxalate until sulfurtrioxide vapours were developed (Kassa *et al*., 2014). The analysis of the heavy metals was carried out as per the procedure described in WHO guidelines using atomic absorption spectrophotometer.

2.7 *In vitro* antioxidant activity

2.7.1 DPPH radical scavenging activity

The free radical scavenging activity of the different extracts and polyherbal formulation was measured using DPPH, employing the method of Blois (1958). One ml of extract and the reference compound in various concentrations (10, 25, 50, 75 and 100 µg/ml) were added to 1 ml of 0.1 mm solution of DPPH in methanol. After 30 min, absorbance was measured at 517 nm. A 0.1 mm solution of DPPH in methanol was used as control, whereas ascorbic acid was used as a reference material (Gupta *et al*., 2007). All tests were performed in triplicate. Per cent inhibition was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

A control = Absorbance of control reaction and

A test = Absorbance of sample equation (Mathangi and Prabhakaran, 2013).

3. Results

3.1 Extraction

The percentage yield of the three extracts were found to be 5.5, 6.5 and 4.2 for AH, BH and AM, respectively.

Table1: Percentage yield

Solvents used	% yield
Hydroalcoholic extract (70:30)	5.5
Hydroalcoholic extract (70:30)	6.5
Hydroalcoholic extract (70:30)	4.2

3.2 Phytochemical analysis of individual and combination of extract

Table 2: Phytochemical analysis of individual and combination of extract

Phytochemicals	Results			
	AH	BH	AM	ABA PHF
Carbohydrates	+	+	+	+
Glycosides	+	+	+	+
Fixed oils and fats	+	-	-	-
Proteins and amino acids	-	+	+	+
Saponins	+	-	+	+
Phenolic compounds	+	+	+	+
Phytosterol	+	+	+	+
Alkaloids	+	+	+	+
Flavanoids	+	+	+	+

3.3 Physicochemical parameters of individual and combination of extract

Table 3: Physicochemical parameters of individual and combination of extract

Parameters	Results			
	AH	BH	AM	PHF
Total ash value	8.3	7.87	8.29	6.89
Acid insoluble ash	1.23	2.56	1.33	2.35
Water soluble ash	5.56	3.59	4.21	3.12
Water soluble extractive value	26.8	24.2	21.8	25.4
Alcohol soluble extractive value	18.42	12.52	16.57	17.14
Moisture content	7.23	4.87	7.42	6.89

3.4 FTIR

3.4.1 *A. heterophyllum*

The spectrum of AH shows peaks at 3422 cm^{-1}, 2921 cm^{-1}, 2101 cm^{-1}, 1635 cm^{-1}, 1468 cm^{-1}, 1381 cm^{-1}, 1093 cm^{-1}, which corresponds to specific rotations around carbon atoms. The peak at 3422 cm^{-1} corresponds to N-H stretching, 2921 cm^{-1} corresponds to CH_2 asymmetric stretching (aromatic), 2301 cm^{-1} corresponds to C-h stretching (aldehyde), 1613 cm^{-1}corresponds to C = N asymmetric stretching vibration, 1468 cm^{-1}corresponds to C-N stretching, 1381 cm^{-1}corresponds to C = C stretching, 1043 cm^{-1}corresponds to C-C asymmetric stretching vibration shows FTIR spectrum of PHF.

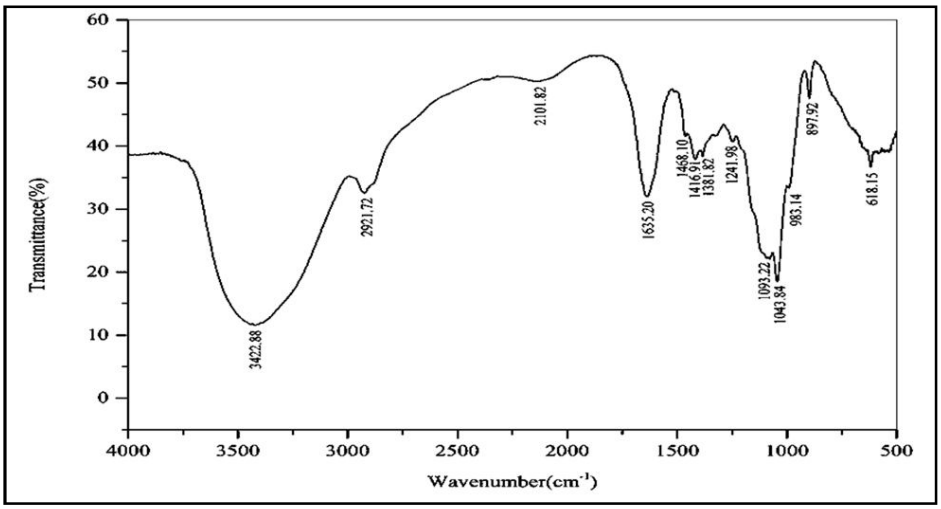

Figure 1: FT-IR sample A.

3.4.2 *B. hispida*

The spectrum of BH shows peaks at 3422 cm^{-1}, 2932 cm^{-1}, 2867 cm^{-1}, 1738 cm^{-1}, 1639 cm^{-1}, 1455 cm^{-1}, 1375 cm^{-1}, 1048 cm^{-1} which corresponds to specific rotations around carbon atoms. The peak at 3422 cm^{-1}corresponds to N-H stretching, 2932 cm^{-1}corresponds to CH_2 asymmetric stretching (aromatic), 2867cm^{-1}corresponds to C-h stretching (aldehyde) , 1738 cm^{-1}corresponds to C=O stretching vibration, 1639 cm^{-1}corresponds to C=N asymmetric stretching vibration, 1455 cm^{-1} corresponds to C-N stretching asymmetric stretching vibration, 1375 cm^{-1}corresponds to C=C stretching, 1048 cm^{-1}corresponds to C-C shows FTIR spectrum of PHF.

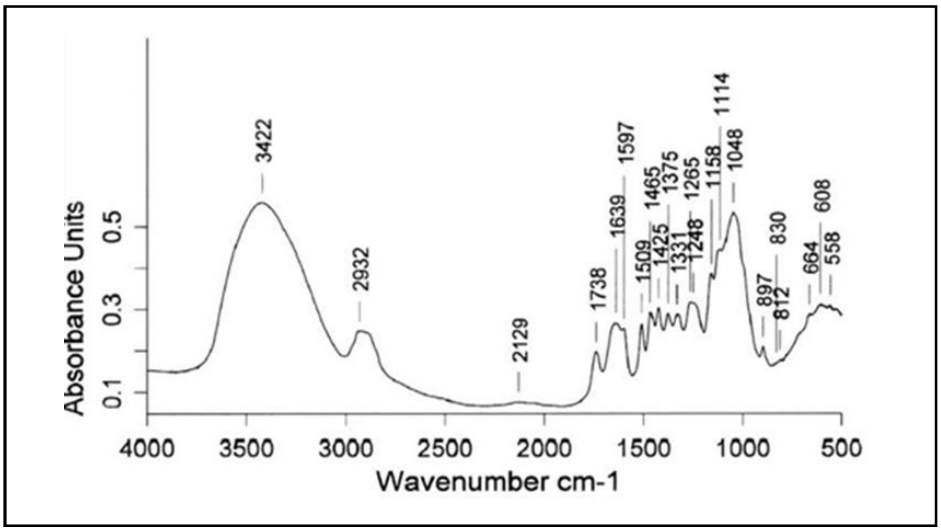

Figure 2: FT-IR for sample B.

3.4.3 *A. marmelos*

The spectrum of AM shows peaks at 3488 cm^{-1}, 2928 cm^{-1}, 2385 cm^{-1}, 1729 cm^{-1}, 1648 cm^{-1}, 1454 cm^{-1}, 1338 cm^{-1}, 1062 cm^{-1}, which corresponds to specific rotations around carbon atoms. The peak at 3488 cm^{-1}corresponds to N-H stretching, 2928 cm^{-1}corresponds to CH_2 asymmetric stretching (aromatic), 2385cm^{-1}corresponds to C-h stretching (aldehyde), 1729 cm^{-1} corresponds to C=O stretching vibration, 1648 cm^{-1} corresponds to C=N asymmetric stretching vibration, 1454 cm^{-1} corresponds to C-N stretching asymmetric stretching vibration, 1338 cm^{-1}corresponds to C=C stretching, 1062 cm^{-1}corresponds to C-C shows FTIR spectrum of PHF.

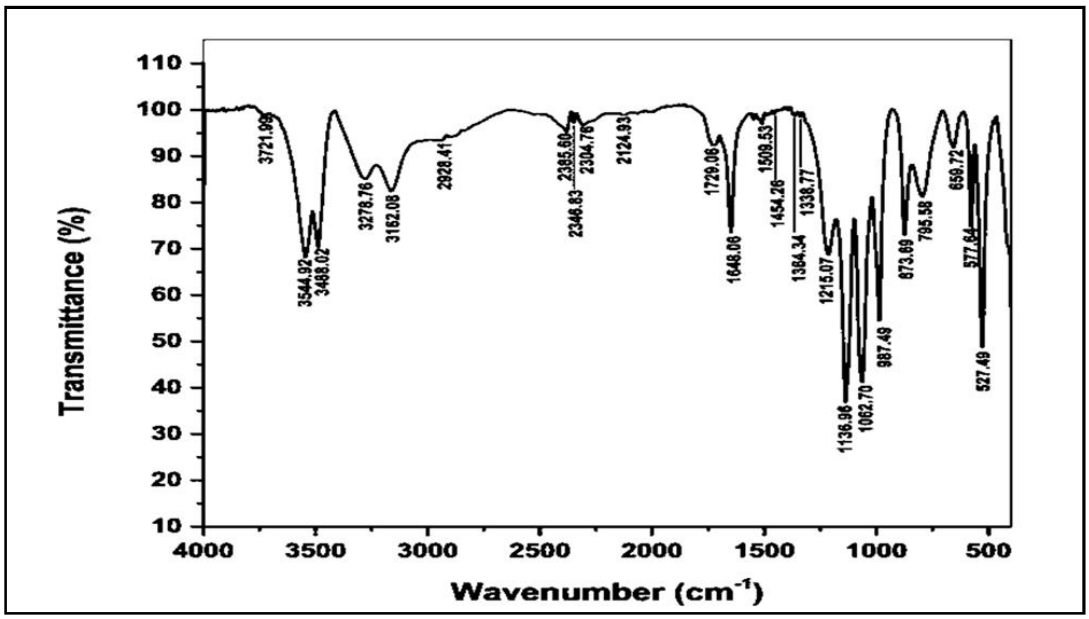

Figure 3: FT-IR for sample C.

3.5 FTIR for polyherbal formulation

The spectrum of PHF shows peaks at 3444 cm^{-1}, 2930 cm^{-1}, 2867cm^{-1}, 1719 cm^{-1}, 1622 cm^{-1}, 1013 cm^{-1}, 1440 cm^{-1}, 1371 cm^{-1} which corresponds to specific rotations around carbon atoms. The peak at 3444 cm^{-1}corresponds to N-H stretching, 2930 cm^{-1} corresponds to CH_2 asymmetric stretching (aromatic), 2867 cm^{-1} corresponds to C-hstretching (aldehyde), 1719 cm^{-1} corresponds to C=O stretching vibration, 1622 cm^{-1} corresponds to C=N asymmetric stretching vibration, 1031 cm^{-1} corresponds to C-C asymmetric stretching vibration , 1440 cm^{-1} corresponds to C-N stretching, 1371 cm^{-1} corresponds to C=C stretching shows FTIR spectrum of PHF.

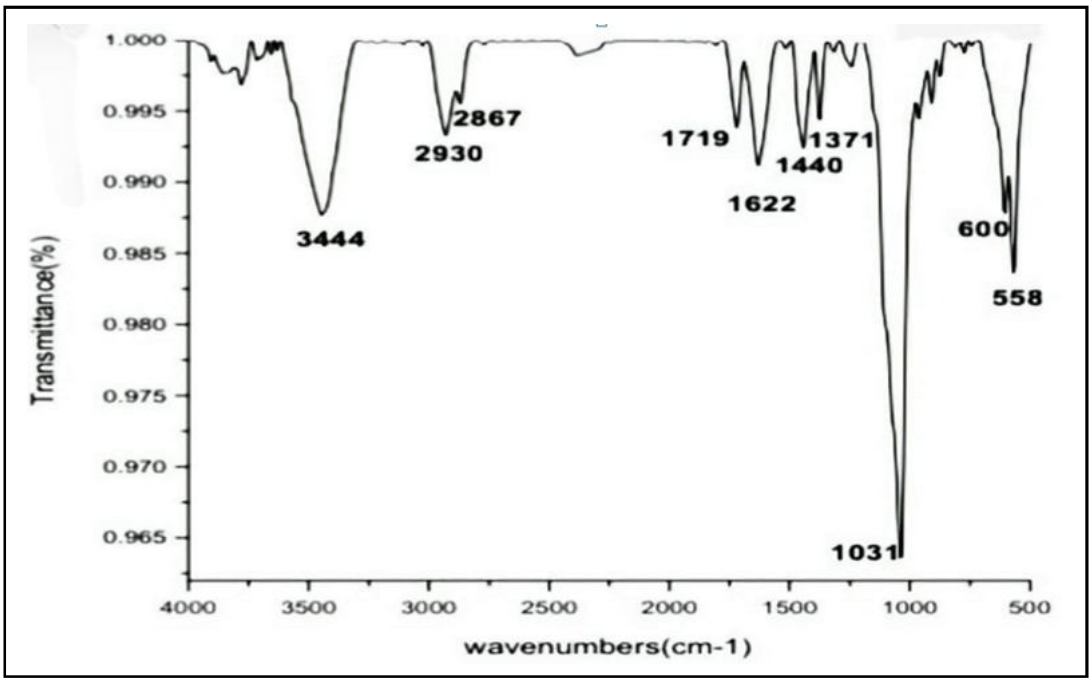

Figure 4: FT-IR for PHF.

3.6 Atomic absorption spectroscopy

Heavy metal contents in medicinal plants depend on climatic factors, plant species, air pollution and other environmental factors. The *A. heterophyllum*, *B. hispida*, *A. marmelos*, polyherbal formulation of ABA showed the presence of arsenic, mercury, lead, nickel, cadmium, chromium, manganese, zinc, iron, aluminium, barium, selenium and copper.

Table 4: Atomic absorption spectroscopy

Heavy metals	AH (ppm)	BH (ppm)	AM (ppm)	ABA PHF (ppm)	Stability limit (ppm)
Arsenic	0.2022	2.32	4.28	4.38	10
Mercury	0.1001	BDL	BDL	NIL	5
Lead	5.007	3.56	2.46	2.67	400
Nickel	2.58	1.58	1.38	BDL	5
Cadmium	0.241	0.031	0.240	0.256	3
Chromium	1.051	1.267	BDL	1.567	0.1
Manganese	2.022	1.86	1.73	2.12	0.3
Zinc	14.7	12.8	14.9	21.88	5
Iron	11.6	12.7	14.8	12.4	0.3
Aluminium	0.18	0.009	0.21	0.22	0.05-0.2
Barium	4.11	3.99	3.28	5.23	2
Selenium	2.08	1.93	2.92	3.08	0.025
Copper	9.45	8.43	7.25	10.45	1.3

3.7 *In vitro* antioxidant activity

The present study was carried out to analyse the antioxidant activity of the three plants and polyherbal formulation. The results obtained in this project work clearly showed that the combination extract has best antioxidant effect at a dose of 300 µg/ ml when it was compared with ascorbic acid as the reference standard.

Table 5: *In vitro* antioxidant activity

Concentration	% inhibition				
	AH	BH	AM	PHF	AA
0	0	0	0	0	0
50	14.46	29.5	22.14	29.8	48.7
100	27.85	44.63	31.2	45.4	58.4
150	34.56	53.02	40.26	54.2	73.82
200	41.2	62.4	48.3	63.5	82.55
250	52.34	68.4	54.69	69.2	90.99
300	58.72	71.4	60.4	75.2	96.6

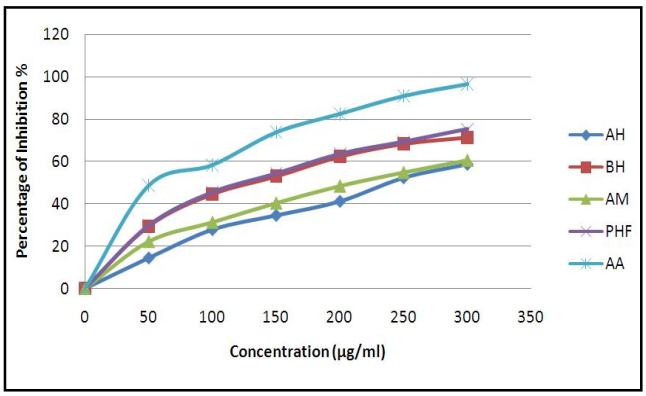

Figure 5: DPPH radical scavengering activity.

4. Discussion

The phytochemical analysis of individual and combination of three extracts shows the presence of carbohydrates, glycosides, proteins, aminoacids, saponins, phenolic compounds, phytosterols and alkaloids (Mohammed *et al*., 2020). The physical parameters, such as loss on drying, ash values and extractive values will be helpful to identify the authenticity of the drug. It serves as a standard data for the quality control of the preparation containing these plants in future.

The FTIR has proved to be an effective instrument for identifying and characterising compounds or functional groups (chemical bonds) (Ibrahim *et al*., 2008). It allows for the qualitative identification of functional groups by observing the appearance of bands in infrared spectrum at a given frequency, which is modified further by the functional groups in the region (Schulz *et al*., 2003). In order to identify the functional groups in all the three extracts and PHF, FTIR was performed. The results indicate the presence of carbonyl groups and aromatic ring structure in all three extracts and PHF. It also shows that the combination of three different herbs in a single formulation was compatible with each other, which is determined from FTIR (Vanshika *et al*., 2022).

A comprehensive analysis of published data indicates that heavy metalsarsenic, mercury, lead, nickel, cadmium, chromium, manganese, zinc, iron, aluminium, barium, selenium and copper occur naturally. In biological systems, heavy metals have been reported to affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei and some enzymes involved in metabolism, detoxification and damage repair (Wang and Shix, 2001). Medicinal plants have been cited as a potential source of heavy metal toxicity to both man and animals (Arruti A *et al*., 2010). The most common heavy metals implicated in human toxicity include lead, mercury, arsenic and cadmium, although aluminium and barium may also cause toxicity. Therefore, world health organization (WHO) recommends that the medicinal plants, which from the raw materials for most herbal remedies, should be checked for the presence of heavy metals.

From the study, the levels of these metals were detected in all three plants and PHF. Presence of heavy metals and minerals in the prepared extracts and PHF were analysed using AAS and were with in the specified limits.

This study determined that the ethanolic extract of all the three plants and PHF showed better antioxidant potential by DPPH radical scavenging method when compared to standard ascorbic acid (Kong *et al*., 2002). So, when compared to the individual extract, the PHF shows more potent antioxidant activity.

5. Conclusion

All the extracts and PHF showed a dose dependent activity. The results confirms the PHF has more potent antioxidant activity when compared with individual extract and herbs in the PHF is compatible with each other which is determined from FTIR. The results from AAS, showed that the tested minerals and metals were within the specified limit, hence it can be safely used for human beings and can also be use for furthur studies. From the above study, we can conclude that PHF possesses promising antioxidant activity which can be considered as a base for further pharmacological evaluation.

Conflict of interest

The author declares no conflicts of interest relevant to this article.

References

Arruti, A.; Ferrandez O. and Irabien, A. (2010). Evaluation of the contribution of local sources to trace metal levels in urban PM 2.5 and PM 10 in the Cantabria region (Northern Spain). J. Environ. Monit., **12**(7):1451-1458.

Balalakshitha, M. and Kolanjinathan, K. (2021). Phytochemical and spectroscopic investigations *Tridax procumbens*. International Journal of Botany Studies, **6**(5):1467-1471.

Blois, MS. (1958). Antioxidant determinations by the use of a stable free radical. Nature, **181**(29):1199-1200.

Chamila, K.P.; Terrence, Madhujit. and Janakie, Eeswara (2020). Bael (*Aegle marmelos* L. Correˆa), a medicinal tree with immense economic potentials. Advances in Agriculture, pp:1-13.

Deepika, B.; Joshi G.; Kumar, R. and Lalit,T. (2014). Phytosociological features and threat categorization of *A. heterophyllum* Wall. ex Royle and *A. ferox* Wall. ex Ser. in Kumaun Himalayan, Journal of Ecology and the Natural Environment, **6**(3):111-118.

Gupta, M.; Mazumder, U.K. and Gomath. P. (2007). Evaluation of antioxidant and free radical scavengering activities of *Plumeria acuminata* L. leaves. Reviews, **7**(8):1361-1367.

Ibrahim, M.; Ali, J.H. and Abrahaham, Jalbout (2008). Molecular spectroscopic study of river Nile sediment in the greater Cariono region. Applied Spectroscopy, **62**(3):306-311.

Kassa, B.; Abi, T. and Tesfahun, K.(2014). Validation of a method for determining heavy metals in some Ethiopian spices by dry ashing using atomic absorption spectroscopy. International Journal of Innovation and Applied Studies, **5**(4):327-332.

Kong, C.; Hu, F. and Xu, X. (2002). Allelopathic potential and chemical constituents of volatiles from *Ageratrum conyzoides* under stress. J. Chem. Ecol., **28**(6):1773-1782.

Mathangi, T. and Prabhakaran, P. (2013). DPPH free radical scavengering activity of the extracts of the Aquatic fern *Marsileaquadrifolia* Linn, International Journal of Current Microbiology and Applied Sciences. **2**(10):534-536.

Mohammed, B.; Kaoutar, E.; Mistafa, E. and Barek, Chaukarad (2020). A comparative study on phytochemical screening, quantification of phenolic contents and antioxidant properties of different solvent extracts from various parts of *Pistacia lentiscus* L., Jornal of King Saud University, **32**(1):302-306.

Muhammad, S.A.; Muhammed, S.L.A. and Anwag, S. (2016). Phytochemical evaluation of polyherbal formulation to identify flavonoids, Pharmacognosy Journal, **8**(6):534-541.

Satyendra, K.Prasad.; Rajkumar, Patel P.K. and Allakh, N. Sahu (2012). Physicochemical standardization and evaluation of *in vitro* antioxidant activity of *Aconitum heterophyllum* Wall., Asian Pacific Journal of Tropical Biomedicine, **2**(2):526-531.

Singh, K.; Saloni, S. and Shalini (2015). Phytochemical screening and TLC profiling of different extracts of leaves, roots and stem of *Aconitum heterophyllum*, a rare medicinal plant of himalayan region. Reviews, **6**(2):194-200.

Schulz, H.; Schrader, B.; Quilitzsch, R.; Pfeffer, S and Kruger, S. (2003). Rapid classification of basil chemotypes by various vibrational spectroscopy methods. Journal of Agricultural and Food Chemistry, **51**(9):2475-2481.

Vanshika, A.; Sumit, G. and Prasad, S.V. (2022). FTIR based rapid microbial quality estimation of fresh cut Jack fruit (*Atrocarpu sheterophyllus*) bulbs. Journal of Food Measurement and Characterization, **16**(3):312-316.

Waidyarathna, S.K.P. and Ediriweera, E.H.S.S. (2020). Therapeutic and culinary uses of *Benincasa hispida*. Journal of Conventional Knowledge Holist Health, **4**(1):1-6.

Wang, S. and Shix. (2001). Molecular mechanisms of metal toxicity and carcinogenes. Mol. Cell biochem., **2**(2):3-9.

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