Evaluation of *in vitro* antioxidant capacity and reducing potential of polyherbal Coded capsules Arthitec 1 and Arthitec 2

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Abstract: Free radicals are partially reduced form of metabolites of Nitrogen and Oxygen. These are highly reactive and potentially toxic compounds which are contributing factors in different chronic disease. The present study was aimed to determine antioxidant capability and reducing ability of coded polyherbal capsules (Arthitec 1 & Arthitec 2). DPPH (2,2'-diphenyl-1-picryl hydrazyl) assay is most commonly used method for gauging antioxidant capability of natural compounds. In this assay DPPH act as stable free radical which react with an antioxidant. For measuring reducing ability suspected antioxidant react with ferric tripyridyltriazine (Fe3± TPTZ) complex and convert ferric into ferrous. Results are evident that both capsule formulations Arthitec 1 & Arthitec 2 have promising antioxidant activity and reducing potential. Antioxidant potential of both coded capsules with varied concentrations (10, 50 and 100 µg/ml) were compared and in both cases scavenging activity and as well as reducing ability raised in a dose dependent manner just like standard Butylated hydroxyanisole (BHA).

Keywords: Poly herbal formulations, antioxidant activity, reducing potential.

INTRODUCTION

In human body, oxidants/antioxidants mechanisms are tightly governed by enzymatic system. Whenever there is any imbalance in this control system, excessive production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) lead to oxidative burden or stress. Prolong overburden of oxidation is causative factor of pathogenesis for example rheumatoid arthritis, diabetes mellitus, neurodegenerative diseases, cardiovascular disease and cancer (Finkel *et al.*, 2000, Valko *et al.*, 2007). Plants and plant extracts have unique anti-oxidant potential to cater this oxidative stress (Alam *et al.*, 2013). Numerous studies revealed the role of natural antioxidant in preventing injuries to body tissues. Flavonoids can prevent injury caused by free radicals through different mechanisms like suppression of reactive oxygen species (ROS), antioxidant enzyme activation, inhibition of α-tocopheryl radicals, suppression of oxidases, Reduce Nitric oxide induce oxidative stress, higher level of uric acid, by intensify antioxidant properties of other low molecular antioxidant (Procházková *et al.*, 2011). In recent time scientists have developed great interest to explore this potential as it could contribute greatly in curing science for chronic diseases.

Basic principle for Antiradical activity assessment or assay was based upon the reduction of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Maximum absorption of DPPH free radicals observed at 515-517 nm due to existence of odd electrons. Pairing off these electrons from a hydrogen donor for example any antioxidant, lead to reduction in strength due to absorption, which change the color. Method of Oyaizu used for determination of reducing ability which based upon transformation of ferric into ferrous state due to antioxidant compounds (Oyaizu, 1986). This method basically relies on increasing absorbance of reaction mixtures. Increase in absorbance is attributed to increasing antioxidant activity or simply increase in absorbance which is directly proportion to increasing antioxidant activity. In addition, colored compounds were resulted by reaction of antioxidants with potassium ferricyanide, trichloro acetic acid and ferric chloride noted at 700nm. Elevation in reaction absorbance depicts the reducing ability of samples (Alam *et al.*, 2013).

MATERIALS AND METHODS

Polyherbal coded capsule Arthitec 1 composed of *Lawsonia inermi, Apium graveolens, Terminalia chebula, Piper nigrum* and *Nigella sativa* while Polyherbal coded capsule Arthitec 2 composed of *Ipomoea turpethum, Apium graveolens, Zingiber officinalis, Colchicum luteum* and *Smilax chinensis.*

Crude drugs were cleaned from foreign matter and earthy material, shade dried, crushed into pieces and soaked overnight in Deionized water separately. After soaking each herb individually, each herb was heated separately in deionized water in 1:10 solute and solvent ratio for 3 hours in steam jacketed vessel. Temperature was

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maintained between 100 to 110°C Each decoction was filtered through mesh no 100 and further concentrated at 90 to 100℃ for 03 hours (time is variable factor depending upon moisture content i.e 2.5%±1.5 for each herb). Moisture content was measured through Karl Fisher reagent. Extract of each herb was obtained in thick paste form.

The resultant pastes were weighed and mixed together along with methyl paraben, propyl paraben and finally adsorbed with microcrystalline cellulose to obtain dry powder.

10% Starch solution was prepared and added to dry powder to obtain damp mass and passed through sieve No. 60 to obtain granules. Granules were spread on tray and dried in hot air oven for 2 hours at 60°C. Magnesium stearate was added and filled into capsules of size zero (0).

Antioxident activity

DPPH radical scavenging activity

DPPH scavenging activity was estimated based upon scavenging ability of 2, 2-Diphenyl-1-(2, 4, 6-trinitrophenyl) hydrazyl (DPPH) radical produced by Arthitec 1 & 2. The method modified by Sadia et al was used to investigate the activity of radical (Shakeel *et al.*, 2015). In this method Freshly prepared 95µl of DPPH solution was mixed with 5 µl 10- 1000 µM in DMSO.

An ethanolic solution of DPPH 2, 2-Diphenyl-1-(2, 4, 6-trinitrophenyl) hydrazyl (M.W= 394.24) (Sigma) of 3mm concentration was prepared. In 96 well plates each well was marked as test, control and blank with respective concentration of test and blank. In subsequent step labelled well were filled with 95µl of DPPH solution. 5 µl of test compound in strength of 10-1000µM in Dimethy Sulfoxide (DMSO) was further added in DPPH solution and this mixture was allowed to mix further. To complete the reaction the 96 well plate was incubated at 37°C for thirty minutes. After thirty minutes at the absorbance of 515nm (Spectramax plus 384 Molecular Device, USA) microtiter plates were observed. Butylated hydroxyanisole (BHA) used as standard. Percentage inhibition was calculated using DMSO as control.

The DPPH radical scavenging activities were determined through following formula

$$\text{DPPH radical scavenging effect (\%)} = Ac - \frac{A_s}{A_c} \times 100 \quad (1)$$

Where
Ac = Control Absorbance
As = Test compound Absorbance

Determination of the reducing power

Phosphate buffer (250µl: pH 6.6: 0.2M) was added to all test compounds separately (100µl: 10-1000µM) prepared in DMSO further a solution of Potassium ferricyanide (250 µl: 1%) was also added to this mixture. Incubation of this mixture was carried out at 50°C in water bath for twenty minutes Moreover, this solution was further subjected to centrifugation for ten minutes at 3000 rpm, 250 µl of top layer of solution was collected after centrifugation in another set of test tubes and equal volume of DMSO (250µl) was also added and allowed to mix. In final step addition of 50µl of 0.1% Ferric chloride to the mixture and the absorbance was noted at 700 nm on spectrophotometer (Specord 2000, Germany) (Alam *et al.*, 2013, Oyaizu, 1986).

$$\text{Percent Reduction Activity} = \frac{A_t}{A_s} \times 100 \quad (2)$$

Where, At = Test Absorbance
As= Standard Capability Absorbance

RESULTS

Results (table 1 & 2) are evident that the both capsules showed good antioxidant activity and reducing ability. When both formulations Arthitec 1 and Arthitec 2 were compared with standard Butylated hydroxyanisole in concentration 10, 50 and 100 µg/ml results showed promising radical scavenging activity. The percent radical scavenging activity increased with increasing concentration just like standard Butylated hydroxyeanisole. Maximum activity 86.5% was observed for Arthitec 1 capsule at concentration 100 µg/ml. Comparably Arthitec 2 capsule showed percentage slightly lower than its counterpart i.e 79.2% at same concentration.

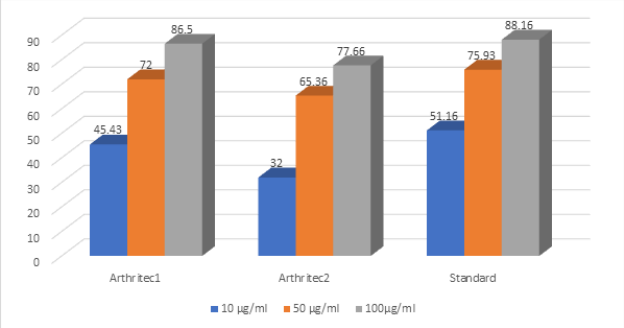

Fig. 1: DPPH radical scavenging activity

In case of reducing potential similar trend, wall followed by both formulations. A significant percentage of reducing potential was observed for both Arthitec 1 and Arthitec 2 capsules when comparison was made with standard Butylated hydroxyanisole at concentration of 10, 50 and 100 µg/ml (table 2).

DISCUSSION

The role of free radicals in pathogenesis of different disease is an established fact (Lawson *et al.*, 2017).

Table 1: DPPH radical scavenging activity

	Concentration tested	Arthretic1 Capsules: Percent Activity (%) ± S.E.M	Arthretic2 Capsules: Percent Activity (%) ±S.E.M	Butylated hydroxyanisole Percent Activity (%) ± S.E.M
1	10 µg/ml	45.43 ± 0.29	32.88 ± 0.65	51.16 ± 0.72
2	50 µg/ml	72.00 ± 0.57	65.36 ± 0.80	75.93 ± 0.52
3	100 µg/ml	86.5 ± 0.76	77.66 ± 0.88	88.3 ± 0.72

Table 2: Determination of the reducing power

	Concentration tested	Arthritec1 capsule: Percent Activity (%) ± S.E.M	Arthritec2 capsule: Percent Activity (%) ± S.E.M	Butylated hydroxy anisole: Percent Activity (%)± S.E.M
1	10 µg/ml	13.3 ± 0.37	12.06 ± 0.17	54.33 ± 0.88
2	50 µg/ml	34.10 ± 0.58	25.50 ± 0.34	68.96 ± 0.54
3	100 µg/ml	56.53 ± 0.48	52.20 ± 0.62	87.36 ± 0.46

Medicinal use of anti-oxidant compounds in treatment of these diseases is a plausible approach and getting acceptance as an emerging way of treatment. (Conforti *et al.*, 2008). Previously synthetic Butyl hydroxyanisole (BHA) and Butyl hydroxy toluene (BHT) were in practice as an antioxidant in different food stuff but contemporarily their use is restricted due to associated risk of carcinogenicity (Basniwal *et al.*, 2009). However, natural antioxidant protects the human body from free radical effects and relieve oxidative stress burden (Pourmorad *et al.*, 2006). Plant contains several compounds which have anti-oxidant properties for instance phenolic compounds, flavonoids, tennis, anthocyanins and carotenoids (Scartezzini *et al.*, 2000). Currently due to long history of use anti-oxidant potential of plants and plant extracts are more valued than ever.

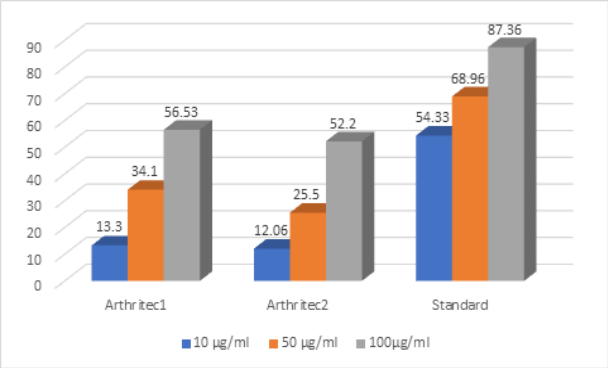

Fig. 2: Determination of the reducing power

DPPH assay could screen a range of compound for anti-oxidant ability, DPPH act as stable free radicle having absorption spectra between 515 nm to 517 nm which could be reduced due to any potential reducing agent (Huang *et al.*, 2005). Reducing potential is usually attributed to reductones which breakdown the free radical chain by donation of hydrogen atom/electron (Elmastas *et al.*, 2006). It has already been reported that polyphenolic and phenolic constituents of plants exhibit antioxidant activity due to their redox properties which enabled them to donate hydrogen and act as reducing agent. The nexus between disease like arthritis and antioxidant have already been reported in number of different studies (Adebayo *et al.*, 2015, Conforti *et al.*, 2008). Exploration of anti-oxidant potential for coded polyherbal formulations Arthitec 1 and Arthitec 2 yielded promising results at concentration of 100 µg/ml which is 86.5% and 77.6% respectively. In smiler manner, reducing potential also increased in dose dependent manner just like standard Butyl hydroxyanesole (BHA). Furthermore, a significant reducing percentage was observed at 100 µg/ml i.e 56.53% and 52.20% for Arthitec 1 and Arthitec 2 respectively.

CONCLUSION

In-vitro antioxidant analysis of coded herbal capsules Arthritec 1 and Arthritec 2 revealed excellent reducing capability and antioxidant ability which might be supportive in management and treatment of stress-related complication associated with arthritis and joint problems.

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