COMPARISON OF IN VITRO ANTIOXIDANT POTENTIAL OF INDIVIDUAL HERBALS AND A NEW GREEN COMBINATION AS AN EFFECTIVE ANTI-OBESITY THERAPY

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Abstract

This study has been carried out with the objective to evaluate the antioxidant potential of individual herbal ingredients of a newly developed PHF. Comparisons of the antioxidant potential were made with the standards by different in vitro antioxidant models such as DPPH assay, ABTS radical scavenging activity, Superoxide (SO) anion scavenging activity assay, Nitric oxide (NO) scavenging assay. Based on our previous findings regarding percentage yield for various solvent extracts, it was found that the yield was high for ethanolic (42%) and water (32%) extracts. Therefore the two extracts were evaluated for antioxidant activity. The in vitro antioxidant activity results confirmed ethanolic extracts showed higher free radical scavenging potential of the PHF against DPPH, ABTS, SO and NO radicals when compared to the aqueous extracts. Also the ethanolic PHF extract showed greater antioxidant activity than the individual herbal drugs. The correlation matrix between the various antioxidant assays, total polyphenolic and flavonoid contents of the ethanolic and aqueous PHF extracts were studied. The results indicated a significant correlation ($p<0.05$ and * $p<0.01$) between the antioxidant assays and the polyphenolic and flavonoidal contents. From the above study, it was implied that the PHF can be used for treating radical-related pathological damage caused due to obesity.*

Key Words: Polyphenols, antioxidant assays, DPPH assay, Superoxide (SO) anion scavenging activity assay, PHF.

INTRODUCTION

Obesity is usually associated with increasing level of oxidation [1,2]. The free radicals in excess cause cumulative damage to the body and antioxidants are adequate enough to neutralize them [3]. Overproduction of free radicals contributes to hundreds of disorders such as atherosclerosis, cancer, diabetes mellitus, arthritis etc. in humans [4]. Chronic oxidative stress in obesity favors the development of end organ damage mainly due to atherosclerosis and non-alcoholic hepatic steatosis [5].

In the past few decades, there has been increasing inclination towards use of herbal drugs against different diseases [6,7]. According to World Health Organization (WHO) estimates, man in developed and

(80%) developing depend on traditional medicinal systems for their basic health care needs [8, 9]. Even treatment of obesity by herbals and their products are presently gaining momentum worldwide, not only because they are inexpensive but also because of their equivalent efficacy. They contain various phytoconstituents with potential properties that can lead to weight loss. Treatments with herbal extracts or functional food ingredients are fast becoming a promising approach to prevent adiposity. Therefore, focus is now on exploring and screening herbals and their different forms of preparations because of their minimal side effects.

Some of the plants used for the treatment of obesity are *Allium sativum* (Garlic), *Anacardium occidentale* (Cashew nut), *Capsicum frutescens* (Red pepper), *Chicorium intybus* (Chicory), *Foeniculum vulgare* (Fennel), *Panax quinquefolium* (American ginseng), *Zingiber officinale* (Ginger), *Thymus vulgaris* (common thyme), *Commiphora mukul* (Guggul), *Coriandrum sativum* (Coriander), *Garcinia cambogia* (Malabar Tamarind), *Linum usitatissimum* (Flax), *Lagenaria siceraria* (Bottle gourd) etc. These plants contain phytoconstituents such as inulin, saponins, tannins, dietary fibres, flavonoids, polyphenols etc that show potential antiobesity properties by inhibiting platelet aggregation, enhancing antioxidant activity, regulation of appetite and fat metabolism, reducing serum lipids etc.

In our study four medicinal plants were chosen for PHF preparation based on their wider availability at affordable costs and also because many previous researchers have reported their strong antioxidant potentials; *P. emblica* [10], *M. uniflorum* [11], *C. longa* [12], *P. zeylanica* [13]. This study can also provide a possible scientific basis for the extensive usage of these four herbals as a combination for functional foods, food supplements, drug intervention; therapies to patients as well as to healthy people.

MATERIALS AND METHODS

Poly herbal formulation extracts for evaluation of antioxidant activity

Percentage yield obtained for different solvent extracts of PHF (petroleum ether, chloroform, ethanol, water) was calculated and it was identified that ethanolic and water extracts gave maximum yield of 42 % and 32 % respectively. Therefore, the two extracts were considered for evaluation of antioxidant activity using different antioxidant methods such as DPPH, Super Oxide, Nitric Oxide and ABTS. Also the synergy of the individual herbal extracts and their capacity to act as free radical antagonists was tested using the *in vitro* antioxidant models.

Evaluation of *in vitro* free radical scavenging activity

The *in vitro* antioxidant activity was measured using various antioxidant models such as: DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay, ABTS (2,2'-aminobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium), Nitric Oxide (NO), Super Oxide (SO) according to the methods described by Blois [14), Rice Evans *et al.,* [15], Garrat [16] and Nishimiki *et al* [17] respectively. The absorbance for free radical scavenging activity measured for respective assays was as follows: DPPH at 517 nm, ABTS at 745 nm, Nitric Oxide at 546 nm and Super Oxide at 560 nm. The

following equation was used to calculate the scavenging ability of DPPH, ABTS, NO and SO radicals:

% inhibition= control - test/ control x 100

Where 'control' was the absorbance of the control reaction and 'test' was the absorbance in presence of extract. The mean values were calculated from three experiments and expressed as SD ± mean. The positive controls were those using the standards, gallic acid (DPPH), rutin (ABTS), ascorbic acid (NO) and quercetin (SO).

Statistical analysis

All the experiments for total phenol and free radical scavenging activity were carried out in triplicate (n = 3) and the results are expressed as mean ± standard deviation (SD). Linear regression analysis using Graph Pad Prism 5.01 was used to calculate IC_{50} and to verify correlations between antioxidant parameters and total polyphenols and flavonoid content.

RESULTS

DPPH scavenging assay (Ethanolic extract)

The IC_{50} value of the standard gallic acid is 8.70 ± 0.10 (8.614x + 47.44, R^2=0.993), scavenging activity of *M. uniflorum*, *P. emblica*, *P. zeylanica*, *C. longa* are 12.5± 1.70 µg/ ml (12.7x + 31.1, R^2= 0.977), 10.41 ± 0.25 µg/ml (11.4x+38.86, R^2=0.968), 11.36 ± 1.00µg/ ml (8.8x+42, R^2=0.986) and 10.86 ± 1.12 µg/ ml (10.6x + 38.6, R^2=0.964) respectively. The IC_{50} value of PHF (10.00 ± 0.53 µg/ml, 8.8x + 42, R^2=0.986) suggests a potential free radical scavenging activity (Figure 4.1i).

The % inhibition vs concentration of sample was plotted. All data were taken in triplicates and expressed as mean ± S D (n=3). Linear regression was

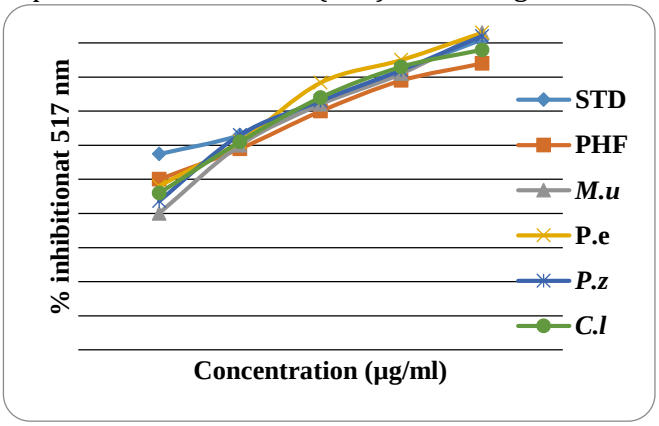


Figure 4.1i. DPPH free radical scavenging activity (ethanolic extract)

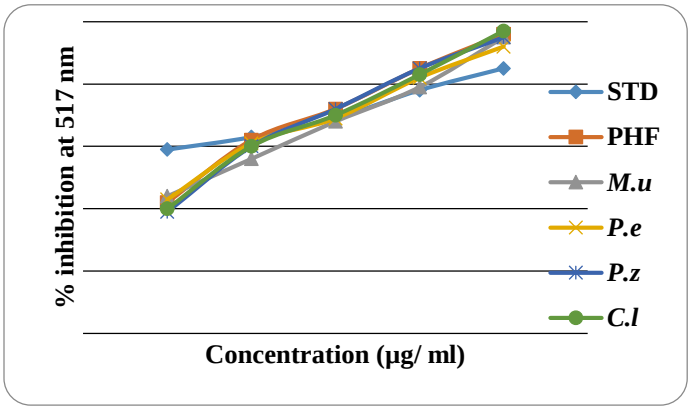
determined and ***p<0.001 was considered significant. Increasing percentage of inhibition between the herbal drugs and PHF is shown as follows: PHF> *P.e* (*P. emblica* > *C.l* (*C. longa*) >*P.z* (*P. zeylanica*)> *M.u* (*M. uniflorum*).

DPPH scavenging assay (Aqueous extract)

The IC_{50} value of the standard gallic acid is 8.47 ± 0.10 (6.7x+50.7, R^2=0.982), scavenging activity of *M. uniflorum*, *P. emblica*, *P. zeylanica*, *C. longa* are 11.36± 1.70 µg/ ml (12.5x + 30.9, R^2= 0.995), 11.60 ± 0.25 µg/ ml (11.9x + 33.7, R^2=0.985), 12.8 ± 10.00 µg/ ml (13.7x + 29.1, R^2=0.980) and 12.5 ± 1.12 µg/ ml (13.7x + 28.9, R^2=0.988). The IC_{50} value of PHF (11.90 ± 0.53 µg/ ml, 13.1x + 32.1, R^2=0.984) suggests a potential free radical scavenging activity (Figure 4.1ii).

Figure 4.1 ii. DPPH free radical scavenging activity (aqueous extract)

The % inhibition vs concentration of sample was plotted. All data were taken in triplicates and expressed as mean ± S D (n=3). Linear regression was



determined and ***p<0.001 was considered significant. Increasing percentage of inhibition between the herbal drugs and PHF is shown as follows: *M.u* > *P.e* > PHF> *C.l* > *P.z*.

ABTS scavenging assay (Ethanolic extract)

The IC $_{50}$ of the standard (rutin) is 14.92 ± 1.24 µg/ ml (0.285x + 60.9, R^2=0.996). Scavenging activity of *M. uniflorum*, *P. emblica*, *P. zeylanica*, *C. longa* are 16.94± 1.02 µg/ ml (0.43x + 48.6, R^2= 0.987), 17.80 ± 0.05 µg/ ml (8.4x + 49.4, R^2=0.986), 20.8 ± 0.12 µg/ ml (10.5x + 39.4 R^2=0.989) and 19.23 ± 0.12 µg/ ml (8.5x + 45.1, R^2=0.990). Ethanolic extract of PHF (16.39 ± 0.04 µg/ml, 6.1x + 53.5, R^2=0.982) showed good antioxidant activity (Figure 4.2 i).

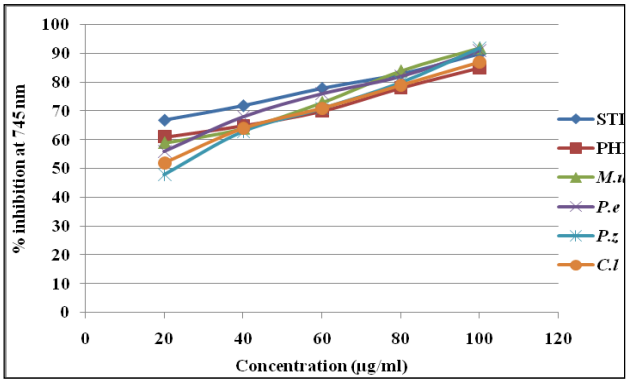


Figure 4.2 i. ABTS radical scavenging activity (ethanolic extract)

The % inhibition vs concentration of sample was plotted. All data were taken in triplicates and expressed as mean ± S D (n=3). Linear regression was determined and ***p<0.001 was considered significant. Increasing percentage of inhibition between the herbal drugs and PHF is shown as follows: PHF > *M.u* > *P.e* > *C.l* >*P.z*.

ABTS scavenging assay (Aqueous extract)

The IC $_{50}$ of the standard (rutin) is 16.6 ± 1.24 µg/ ml (7.3x+54.3, R^2=0.985). Scavenging activity of *M. uniflorum*, *P. emblica*, *P. zeylanica*, *C. longa* are 22.2 ± 1.02 µg/ ml (11.8x + 35.4, R^2= 0.987), 23.25 ± 0.05 µg/ ml (11.8x + 33.8, R^2=0.987), 21.27 ± 0.12 µg/ ml (10.9x + 39.1 R^2=0.978) and 20.00 ± 0.12 µg/ ml (9.2x + 42, R^2=0.994). Aqueous extract of PHF (20.00 ± 0.04 µg/ ml, 8.80x + 42.2, R^2=0.993) showed good antioxidant activity (Figure 4.2 ii).

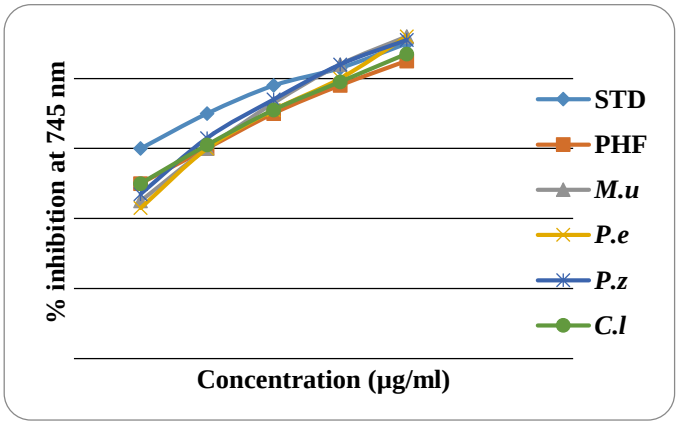


Figure 4.2 ii. ABTS radical scavenging activity (aqueous extract)

The % inhibition vs concentration of sample was plotted. All data were taken in triplicates and expressed as mean ± S D (n=3). Linear regression was determined and ***p<0.001 was considered significant. Increasing percentage of inhibition

between the herbal drugs and PHF is shown as follows: PHF = *C.l* > *P.z*> *M.u*> *P.e* .

Superoxide radical scavenging activity (Ethanolic extract)

The IC_{50} value of the standard (quercetin) is 8.62 ± 1.35 µg/ ml (9.2x + 46.8x, R^2= 0.987). Scavenging activity of *M. uniflorum*, *P. emblica*, *P. zeylanica*, *C. longa* are 15.01 ± 1.02 µg/ ml (9.83x + 22.78, R^2= 0.988), 10.20 ± 0.05 µg/ ml (9.6x+39, R^2=0.981), 13.15 ± 0.52 µg/ ml (14.4x + 29.4 R^2=0.982) and 9.8 ± 0.62 µg/ ml (11x + 40.6, R^2=0.990). Ethanolic extract of PHF (9.43 ± 0.04 µg/ ml, 10.5x + 40.3, R^2=0.989) showed good antioxidant activity (Figure 4.3i).

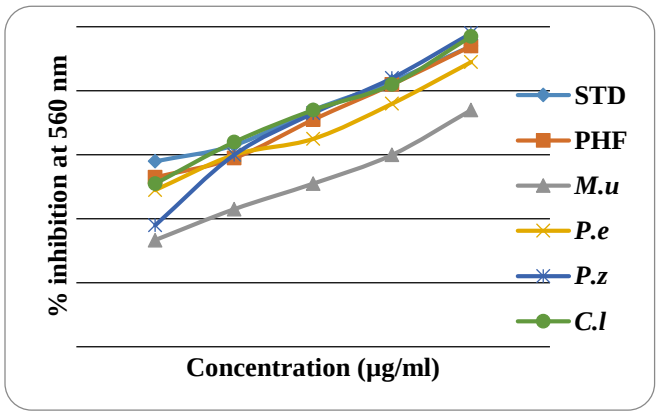


Figure 4.3 i. Super Oxide radical inhibition activity (ethanolic extract)

The % inhibition vs concentration of sample was plotted. All data were taken in triplicates and expressed as mean ± S D (n=3). Linear regression was determined and ***p<0.001 was considered significant. Increasing percentage of inhibition between the herbal drugs and PHF is shown as follows: PHF> *C.l* > *P. e*> *P.z* >*M.u*.

Superoxide radical scavenging activity (Aqueous extract)

The IC_{50} value of the standard (quercetin) is 10.41±1.02 µg/ ml (8.1x + 45.1, R^2= 0.992). Scavenging activity of *M. uniflorum*, *P. emblica*, *P. zeylanica*, *C. longa* are 20.00 ± 0.62 µg/ ml (14.1x + 27.3, R^2=0.983), 19.20 ± 0.05 µg/ ml (14.2x + 23.6, R^2=0.990), 21.73 ± 0.52µg/ ml (11.1x + 20.7 R^2=0.984) and 19.00 ± 0.04 µg/ ml (11.3x+36.1, R^2=0.986). Aqueous extract of PHF (20.83 ± 0.04 µg/ ml, 15.9x + 19.5, R^2=0.981) showed good antioxidant activity (Figure 4.3ii).

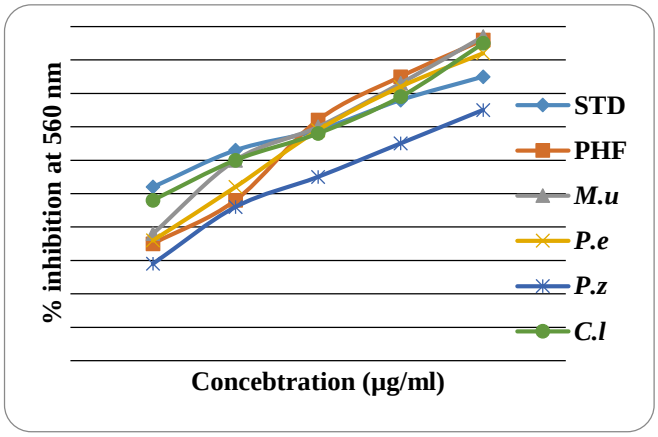


Figure 4.3 ii. Super Oxide radical inhibition activity (aqueous extract)

The % inhibition vs concentration of sample was plotted. All data were taken in triplicates and expressed as mean ± S D (n=3). Linear regression was determined and ***p<0.001 was considered significant. Increasing percentage of inhibition between the herbal drugs and PHF is shown as follows: *C.l* >*P.e*> *M.u* > PHF>*P.z*.

Nitric Oxide scavenging assay (Ethanolic extract)

The IC_{50} value of the standard (ascorbic acid) is 7.69 ± 1.03 µg/ ml (8.248x + 57.70, R^2= 0.996). Scavenging activity of *M. uniflorum*, *P. emblica*, *P. zeylanica*, *C. longa* are 9.43 ± 1.02 µg/ ml (8.4x + 44.2, R^2= 0.986), 8.47 ± 0.55 µg/ ml (10x+47, R^2=0.990), 10.00± 0.76 µg/ ml

(10.1x + 41.31, R^2=0.991) and 9.09 ± 0.98 (9.7x + 44.1, R^2= 0.989). Nitric oxide free radical scavenging assay activity of PHF showed good antioxidant activity (8.06 ± 1.2µg/ ml (8.5x + 51.9 R^2=0.988) (Figure 4.4 i).

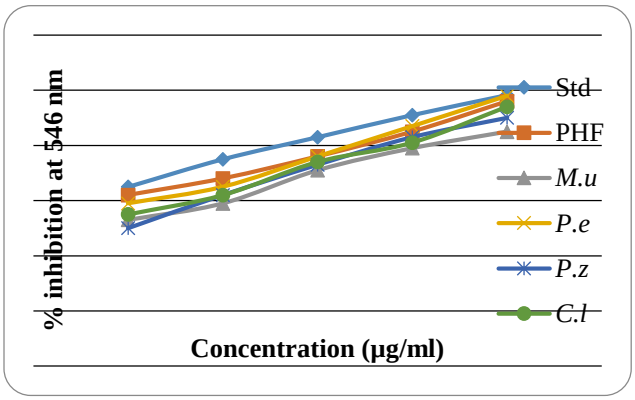


Figure 4.4 i. The percentage of Nitric Oxide radical inhibition activity (ethanolic extract)

The % inhibition vs concentration of sample was plotted. All data were taken in triplicates and expressed as mean ± S D (n=3). Linear regression was determined and $^{***}p<0.001$ was considered significant. Increasing percentage of inhibition between the herbal drugs and PHF is shown as follows: PHF> *P.e* > *C.l*.> *M.u*> *P.z*

Nitric Oxide scavenging assay (Aqueous extract)

The IC_{50} value of the standard (ascorbic acid) is 10.63 ± 1.03 µg/ ml (9.4x + 39.6, R^2= 0.987). Scavenging activity of *M. uniflorum*, *P. emblica*, *P. zeylanica*, *C. longa* are 19.23 ±1.02 µg/ ml (14.7x + 21.7, R^2= 0.995), 11.62 ± 0.55 µg/ ml (12.8x + 29.2, R^2=0.991), 20.8 ± 1.2µg/ ml (9.1x + 28.9 R^2=0.996) and 14.76 ± 0.76 µg/ ml (14.9x + 24.1, R^2=0.970). Nitric oxide free radical scavenging assay activity of PHF showed good antioxidant acivity (18.18 ± 0.54 µg/ ml, 13.4x + 30, R^2= 0.987) (Figure 4.4ii).

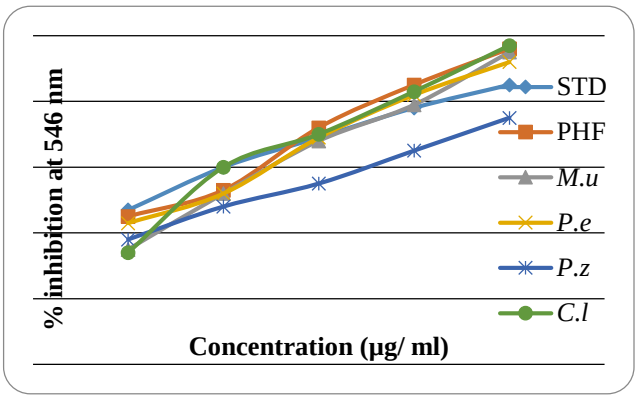


Figure 4.4 ii. The percentage of Nitric Oxide radical inhibition activity (aqueous extract)

The % inhibition vs concentration of sample was plotted. All data were taken in triplicates and expressed as mean ± S D (n=3). Linear regression was determined and $^{***}p<0.001$ was considered significant. Increasing percentage of inhibition between the herbal drugs and PHF is shown as follows: *P.e* > *C.l* > PHF> *M.u*. > *P.z*. The correlations between the various antioxidant assays and the total polyphenolic and total flavonoid content of the ethanolic extracts (Figure 4.5) and aqueous extracts (Figure 4.6) was studied and the significance of the correlations were expressed as $^{*}p<0.05$ and $^{**}p<0.01$ respectively.

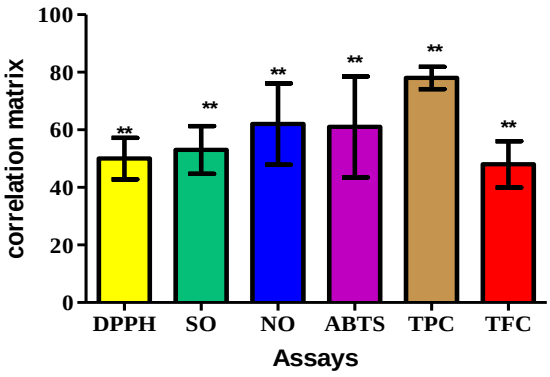


Figure 4.5: Correlation matrix between antioxidant properties and polyphenolic contents of PHF (ethanolic extracts)

Symbol ** represents p value: ** p> 0.01 for each assay. Free radical scavenging activity (% inhibition of DPPH, ABTS, NO and SO), TPC: total polyphenol content (mg GAE mg^{-1}), and TFC: total flavonoid content (mg QE mg^{-1}). R^2 value range was between 0.965 to 0.998.

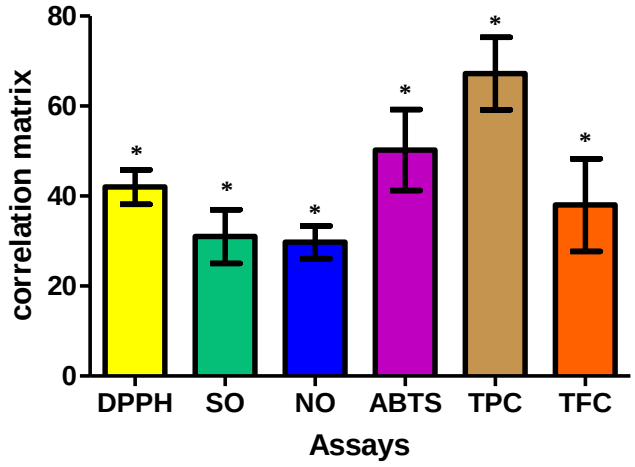


Figure 4.6: Correlation matrix between antioxidant properties and polyphenolic contents of PHF (aqueous extracts)

Symbol * represents p value: *p<0.05 for each assay. Free radical scavenging activity (% inhibition of DPPH, ABTS, NO and SO), TPC: total polyphenol content (mg GAE mg^{-1}), and TFC: total flavonoid content (mg QE mg^{-1}). R^2 value range was between 0.961to 0.999.

DISCUSSION

Obesity is linked to chronic inflammatory diseases characterized by higher than normal oxidative stress [18]. There is a close association between obesity and oxidative stress. Phytochemical screening of ethanolic and aqueous extracts of PHF, confirmed the presence of polyphenolic compounds. Quantitative analysis by HPTLC of the ethanolic extract of the PHF also showed that it possessed a significant amount of phenolic compounds. The detected compounds were quercetin, rutin, apigenin, gallic acid, curcumin and plumbagin all of which belong to the class of polyphenols.

All the four plants used in this study have been reported to exhibit great antioxidant property due to high polyphenolic content. The ethanolic extracts of the individual herbals showed better inhibitory activity than the aqueous extracts. The percentage inhibition of *M. uniflorum*, *P. emblica*, *C. longa* and *P. zeylanica* against DPPH radicals were found to be 40%, 48 %, 46 % and 44 % respectively at minimum inhibitory concentration (IC_{50}) of 10 µg/ ml. In ABTS radical scavenging assay, IC_{50} values were observed as 59 % (*M. uniflorum*), 56 % (*P. emblica*), 52 % (*C. longa*) and 48 % (*P. zeylanica*) at minimum concentration of 20 µg/ ml. IC_{50} values of 33.3 % (*M. uniflorum*, at 30 µg/ ml), 49 % (*P. emblica*, 10 µg/ ml), 51 % (*C. longa*, 10 µg/ ml) and 38 % (*P. zeylanica*, 10 µg/ ml) was observed in Super Oxide (SO) radical scavenging assay. In Nitric Oxide (NO) radical scavenging assay, IC_{50} values were observed as 53 % (*M. uniflorum*), 59 % (*P. emblica*), 55 % (*C. longa*) and 50 % (*P. zeylanica*) at minimum concentration of 10 µg/ ml. Hence they were used for the preparation of PHF which can play a major role in eradicating free radical mediated injury.

From the present study it was concluded that the composite herbals exhibited antagonistic action against different free radicals. *In vitro* antioxidant activity results of the PHF confirmed its free radical scavenging potential against DPPH, ABTS, SO and NO reactive species. In the ABTS scavenging assay, it was very well observed that the ABTS radicals,

distinguished as a blue coloured chromophore was reduced to ABTS on increasing concentrations. The results were compared with the standard rutin and individual herbals and the values demonstrated the ethanolic PHF extracts as potent antioxidant with their values following the order PHF > *M. uniflorum* > *P. emblica* > *C. longa* >*P. zeylanica*. The effect of inhibitory assay of DPPH radical furthermore substantiated the fact that the extracts effectively act as antioxidants as it followed the order PHF >*P.emblica* >*C. longa* >*P. zeylanica* > *M.uniflorum*. This suggests that the ethanolic extract of the PHF contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity [19]. The ability of the ethanolic PHF and the reference compound quercetin to quench SO radicals from reaction mixture is indicated in the decrease of the absorbance at λ 560 nm. SO anions are also harmful reactive radical species that damages cellular components in biological systems [20]. From the results it can be put forward that the ethanolic PHF extracts are more potent scavenger of SO radicals than the individual herbal used with a decreasing order of PHF > *C. longa* > *P. emblica* > *P. zeylanica* >*M. uniflorum*. The NO scavenging activity was performed and compared with the standard ascorbic acid. The results of scavenging activity of Nitric Oxide indicated lower levels of the NO radicals formed which may be due to the antioxidant principles present in the PHF extract and is observed in the order PHF > *P. emblica* > *C. l*onga > *M. uniflorum* > *P. zeylanica*. This may be due these antioxidant principles may have competed with oxygen to react with nitric oxide which then formed nitrite anions, thereby inhibiting the generation of nitrite [21, **22**]. It was notable that the ethanolic PHF extracts showed a significantly higher antioxidant activity (% inhibition against radicals in the order NO> ABTS > DPPH > SO respectively) than the aqueous extracts in the order DPPH> NO > SO > ABTS (Figures 4.1i, ii, 4.2i,ii, 4.3i,ii and 4.4i,ii). The above results concluded that the aqueous PHF extracts showed lesser synergy of various phytochemicals than in ethanolic extracts. Also the ethanolic extracts showed more synergy of composite herbals and efficient antagonistic activity to neutralize the free radicals.

The correlation coefficient for ethanolic and aqueous extracts of PHF for total flavonoid, phenolic content with its antioxidant capacity was studied. As shown in figures 4.5 and 4.6, the total flavonoid and phenolic content of ethanolic extract of PHF highly correlated ($^{**}p < 0.01$) with the antioxidant activity than the aqueous extracts ($^{*}p < 0.05$).

Fruits of *P. emblica* contains high amount of ascorbic acid and have been evaluated for antioxidant effects. It is reported that presence of tannins prevents oxidation of ascorbic acid in dried fruits [23]. Recent pharmacological activities on *P. emblica* fruit extracts showed protective action against oxidative stress and ameliorated hepatic lipid peroxidation [24]. Nampoothiri *et al*., [25] reported *in vitro* inhibitory potential of aqueous and methanolic extracts of *P. emblica* fruits against DPPH, NO, SO and hydroxyl radicals and scavenging ability of *P. emblica* fruit extracts was proved against H_2O_2, peroxynitrite, hypochlorous acid and singlet oxygen [21].

Curcuminoids, the phenolic compounds of *C. longa* have been associated to antioxidant activity against DPPH, ABTS and Superoxide anion radical scavenging assays [26, 27]. Curcumin is a potent scavenger of different Reactive Oxygen Species (ROS) including radicals such as nitrogen dioxide, hydroxyl radicals and also shown to inhibit lipid peroxidation in various animal models [28, 27,29].

Ethanolic extracts of *M. uniflorum* showed significant radical scavenging activity by Nitric Oxide, Hydroxyl and DPPH methods [30, 31]. Singh *et al*., [29] showed that the methanolic extracts having high antioxidant activity than the standard ascorbic acid in the DPPH and Hydrogen peroxide radical scavenging environments. The presence of phytoconstituents such as flavonoids and phenolic compounds may be responsible for the above radical scavenging activities [32]. Kim and Lee [33] reported that the antioxidant activity of *M. uniflorum* was due phenolic acid content which can facilitate electron delocalization between the aromatic ring and propenoic acid.

High contents of quercetin, kaempferol, luteolin and other phenolic acids detected in *P. zeylanica* extracts exhibited a significant linear relationship with high antioxidant activity in the ABTS and FRAP assay environment [34, 35, 36].

PHF extracts prepared by Jain and Argal [37] (three drugs *Bryophyllum pinnatum, Syzigium aromaticum & Ocimum sanctum*) and Parasuraman, [38] (extracts of Triglize™ -10 ingredients) for the treatment of obesity, hypertension, palpitation, etc, showed a moderate antioxidant activity by DPPH method. DPPH scavenging effect of extract of N-Miracle PHF was compared to standard ascorbic acid which indicated its DPPH scavenging affectivity compared to ascorbic acid [39]. According to researchers [40, **41**] secondary metabolites, mainly polyphenols represent the major components with stronger antioxidant capacity. Correlations are exhibited among antioxidant properties and polyphenols and flavonoid contents, which were indicated from their study on the antioxidant activity of their polyherbal formulation (*Tinospora cordifolia, Withania somnifer*a and *Emblica officinalis*. *P. emblica* contains phenolic acids, which have been proved for their multiple biological effects including antioxidant activity [42, 12, 43, 44]

Studies on the phenolic acids metabolism established the fact that these compounds are located in the membrane at the interface lipid/water and are the first to react with the ROS formed in these areas [45, **46**]. The ability of enzymes to inhibit and its antioxidant capacity depends on the type of conjugation during biotransformation and their location in the body. As part of this, they can act directly by scavenging free radicals and donating hydrogen or electrons (additive effect) resulting in more stable compounds, or compounds that can stabilize compounds obtained from free radicals [47].

Free radicals can generate oxidative damage to biomolecules such as proteins, DNA and lipids and eventually cells and tissues. The antioxidants can prevent these oxidative damages and increased intakes from the diet will reduce the risks of chronic diseases; improves oxidative stress [48].

A huge volume of research work is needed to link natural antioxidants and treatment at the same time to

find drugs free of side effects to the maximum. Hence, it has become essentially important to search for new natural antioxidants based on natural formulations [49, 50]. This has led to the need for developing a product of natural origin with therapeutic potentials against obesity and in turn controls serious implications.

Conclusion

The PHF showed a better antioxidant activity when compared to the free radical scavenging activity of the individual ingredients used in the formulation. This suggests the better synergy of all the plants used for the PHF. In conclusion, this newly developed formulation can serve as a potent antioxidant therapeutic product after its proven *in vitro* antioxidant capacity and thereby can reduce or control the secondary complications that can develop as a result of obesity.

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