International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685, Impact Factor: RJIF 5.28
www.pharmacyjournal.net
Volume 2; Issue 1; January 2017; Page No. 01-08

Synergistic action and HPTLC fingerprinting of novel polyherbal formulations containing *Neolamarckia cadamba*

Kawaljit Kaur, Dr. Sugandha S. Shetye, Valsamma Wilson

Department of Chemistry, KJ Somaiya College of Science and Commerce, Vidyavihar, Mumbai, Maharashtra, India

Abstract

In this, four formulations of *Neolamarckia cadamba* with *Butea monosperma, Citrus sinensis, Hibiscus rosa-sinensis,* and *Spinacia oleracea* were evaluated for synergy, on the basis of antioxidant activity by in-vitro testing using DPPH Method, Nitric oxide radical scavenging assay method and Phosphomolybdenum method. Synergy in antioxidant activity of all four formulations was supported by enhancement of the flavonoid and total phenolic content as compared to their values for the single plant(s) drugs. HPTLC study revealed that there was an enhancement of area under the peak for a few R_f values in the formulations that exhibited synergy in their radical scavenging activity. Combination Index values for the formulations were found to be indicative of synergism. The formulations thus offer can desirable pharmacological action with a lower dose with the same or better therapeutic effect.

Keywords: synergy, antioxidant activity, polyherbal formulations**,** combination index, *Neolamarckia cadamba, Butea monosperma, Citrus sinensis, Hibiscus rosa- sinensis, Spinacia oleracea*

1. Introduction

In Ayurveda, herbal medicines are usually mixtures of many constituents, which may have unknown active principles. The scientific evaluation of safety and efficacy of herbal products and medicinal preparation is thus of vital importance from both medicinal and economic perspectives [1], to arrive at the benefits and limitations of the drugs and thereby widen their scope for future use. Depending upon the site of collection and annual growth cycles of the source herbs, there are bound to be chemical and natural differences in quantity of active constituents in herbal materials. A validated method is one that offers an acceptable analytical process for its intended purpose for pharmaceutical testing [2]. Thus choice of the most appropriate analytical method to quantify the compounds most correlated with pharmacological activity or qualitative markers with accompanying chromatograms is desirable for validation.

The present study was aimed at composing novel formulations of *Neolamarckia cadamba (N. cadamba) (N. cad)* with herbal materials known for their antioxidant properties in combination for studying their synergistic action. Pharmacologists have proposed and established that individual action of one drug is subject to modification when combined with a second drug, so that multi-drug regimens ("combination therapy") confer beneficial new actions and therapeutic benefits that do not occur when using each drug on its own [3], with the occasional benefit of fewer deleterious side effects [4].

Antioxidants interact with free radicals and thus terminate their chain reactions before vital molecules are damaged. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues [5]. A balance between free radicals and antioxidants is necessary for proper physiological function. To establish their synergy, antioxidant activity of single plants and their combinations were studied by three different methods, namely; DPPH method, Phosphomolybdenum method and Nitric oxide radical scavenging assay method.

It is observed that the presence of polyphenolic components, which are secondary plant metabolites are responsible for the antioxidant activity exhibited by them [6]. The study of synergistic action of the polyherbal formulations was reinforced by evaluation of total flavonoid and total phenolic contents. High Performance Thin Layer Chromatographic (HPTLC) fingerprinting also substantiated synergistic action of the formulations by showing enhancement in certain peak areas.

A brief overview of the four selected plants is as listed under:

Neolamarckia cadamba			
		Phytoconstituents known	**Used as/ in treatment of**
Family: Rubiaceae	part used - bark	Saponins, indole and quinoline alkaloids, secoiridoids and triterpenes [7, 8, 9, 10].	diabetes mellitus [11] wounds, debility and antimicrobial [12, 13] analgesic, antipyretic, anti - inflammatory [14] hypolipidemic[15]
Butea monosperma			
Family: Fabaceae	part used - flower	Monospermoside (butein 3-e-d-glucoside) and isomonospermoside, flavonoids (palasitrin, prunetin) and steroids, triterpene, butein, butin, isobutrin, coreopsin, isocoreopsin (butin 7-glucoside), sulphurein,[16], chalcones, aurones, lignoceric acid, amino acids, myricyl alcohol, stearic, palmitic, arachidic acids [17].	Leprosy, antifungal activity, anti-inflammatory activity, skin diseases, burning sensation, diarrhea [18].

Citrus sinensis			
		Phytoconstituents known	**Used as/ in treatment of**
Family: Rutaceae	part used - fruit peel	Reducing sugars, saponins, deoxysugars cardiac glycosides, tannins and flavonoids, flavanones, flavones and flavonols, polymethoxylated flavones.	viral and bacterial infections [19] antibacterial, Klebsiella pneumonia
Hibiscus rosa-sinensis			
Family:Malvaceae	part used - flower	Alkaloids, cardenolides and bufadienolides, tannins, protein and carbohydrates, saponin glycosides, flavonoids [20], terpenoids and steroids [21].	Antiseptic for boils and ulcers. Swellings and skin infections [22].
Spinacia oleracea			
Family: Chenopodiaceae	part used- leaves	Flavonoids: querecetin, kampeferol [23]; apigenin; luteolin; patuletin; spinacetin; jaceidin; 5,4'-dihydroxi-3,3'-dimithoxi-6,7-methylene-dioxi- flavone ($C_{18} H_{14} O_{8}$.); 3, 5, 7, 3', 4'pentahydroxi-6-methoxiflavone. Phenolic Compounds: para-coumaric acid, ferulic acid, ortho- coumaric acid [24]. Carotenoids, Vitamins: Spinacia oleracea contains high concentration of vitamin A, E, C, and K. and also folic acid, oxalic acid . Minerals: magnesium, manganese, calcium, phosphorus, iron, zinc, copper and potash. Glucoside, patuletin and spinacetin 3-gentiobiosides [25].	laxative, diuretic, anthelmentic, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, ring worm scabies, leucoderma, scalding urine, arrest vomiting, febrile conditions [26]. CNS depressant, protection against gamma radiation, hepatoprotective properties.

2. Materials and methods

Plant collection and identification

The bark of *N. cadamba* was collected from the premises of K. J. Somaiya College, Vidyavihar, Maharashtra, India. Fresh leaves of *Spinacia oleracea* (*S. oleracia*) (*S. ole*) and flowers of *Hibiscus rosa-sinensis (H. rosa-sinensis) (H. Rosa)* were procured from local sources of Vashi, Navi Mumbai, India. Voucher specimens has been submitted to the the Blatter Herbarium, St. Xavier's College, Mumbai. Flower samples of *Butea monosperma (B. monosperma) (B. mon)* and fruit of *Citrus sinensis (C. sinensis)(C. sin)* procured from Aromatic and Medicinal Plants' project at Rahauri, Maharashtra were authenticated at The Botanical Survey of India, Western Region at Pune, India. 2, 2-diphenyl-1- picrylhyrazyl radical (DPPH) was purchased from Sigma Aldrich while other chemicals, solvents and reagents were from Merck, India.

Preparation of the samples

The bark of *N. cadamba*, leaves of *S. oleracea*, flowers of *H. Rosa-sinensis* and *B. monosperma*, peels from fruits of *C. sinensis* were separately washed under running water thoroughly to remove the dirt and other contaminants, shade dried, ground to a powder and extracted with methanol using Soxhlet extractor. The coloured solution obtained from each plant material was concentrated on a Buchi rotatory evaporator at 40 °C and a reduced pressure and then evaporated to dryness. In case of the formulations, the same extraction procedure was carried out on 1:1 (by mass) intimate mixture of the bark powder of *N. cadamba* and powders of the stated plant parts of the other four specimens.

Phytochemical testing

The five single plants and the four formulations were tested for the presence of secondary plant metabolites such as flavonoids, tannins, steroids, alkaloids, saponins, glycosides, phenolics etc for qualitative screening, using standard phytochemical methods.

Evaluation of free radical scavenging activity by DPPH method

The determination of the radical scavenging activity of the methanol extract was carried out using the DPPH (2, 2-diphenyl-1- picrylhydrazyl) assay. Solutions at a concentration of 100 µg /mL of sample extract in methanol were prepared. DPPH in methanol was added to solution of the extracts and standard, and allowed to stand at room temperature in a dark chamber for 30 min. The colour changed from dark violet to yellow. The absorbance was measured at 514 nm on a UV-VIS spectrophotometer. The decrease in absorbance was then converted to percentage radical scavenging antioxidant activity (% RSA) using the formula.

Percentage radical scavenging activity,

$$\%RSA=100 - \{[(A_{control} - A_{sample}) \times 100] / A_{control}\}$$

Where

$A_{control}$ = Absorbance of the control

A_{sample} = Absorbance of the sample

Blank used was methanol and sample solution, negative control used was DPPH solution with methanol, ascorbic acid was used as a standard.

Evaluation of total antioxidant capacity (TAC) by Phosphomolybdenum method

The total antioxidant capacity of the methanol extract was evaluated by the Phosphomolybdenum method according to the procedure described by Prieto *et al*. (1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid ρH. The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against blank at room temperature. Methanol was used as the blank. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared using ascorbic acid.

Nitric oxide radical scavenging assay

An aqueous solution containing sodium nitroprusside, phosphate buffer saline, and the individual extract and combination or the standard solution was incubated at 25° C for 2.5 h. Thereafter, 0.5mL of the reaction mixture was pipetted out and mixed with sulfanilic acid reagent and allowed to stand for 5 min for the completion of diazotization. Subsequently, 1-Naphthylamine was added, mixed, and allowed to stand for 0.5 h. A pink colored chromophore was formed; the absorbance of this solution was measured at 540 nm against the corresponding

blank solution. Reduction in nitric oxide free radicals was calculated using the formula:

$$\% \text{ Inhibition} = [(A\text{control} - A\text{sample}) / A\text{control}] \times 100$$

Evaluation of total phenolics content using Folin-Ciocalteu reagent

Reduction of phosphomolybdic-phosphotungstic acid (Folin) reagent to a blue-colored complex in an alkaline solution occurs in the presence of phenolic compounds. The absorbance is measured at 765 nm. A mixture of water and reagents is used as a blank. The method measures the number of potentially oxidizable phenolic groups.

Calibration curve was prepared by mixing methanolic solution of gallic acid. All determinations were carried out in triplicate. The total phenolic compound in the extract in gallic acid equivalents (GAE) was calculated.

Evaluation of total flavonoid content by $AlCl_3$ colorimetric method

The extract was added to distilled water taken in a 10 ml volumetric flask. To this 0.30 ml of sodium nitrite was added. After 5 min, 10% aluminium chloride was added and mixed well. After 5 min, 1 M sodium hydroxide was added followed by dilution up to the mark with distilled water. All five extracts of the three individual plants and their two formulations and a set of reference standard solutions of quercetin was prepared in a similar manner. Absorbance of the solutions was measured at 510 nm against the reagent blank using a spectrophotometer.

HPTLC fingerprinting

Pre-coated silica gel aluminium plates 60 F_{254} TLC plates of 0.2 mm thickness and 10x10 cm size were activated at 110° C for five minutes and then spotted with the samples in bands of width 6.0 mm using CAMAG Linomat 5 sample applicator. The solvent systems used were as follows:

3. Results and Discussion

Drug-drug interaction can be synergy; when the effect is totally or partially additive; potentiation; when the effect of one drug is increased by intake of another drug without notable effect or antagonism; in which the effect of one drug is suppressed by another. In Ayurveda, synergistic blends utilize herbs in the following five ways:

1. The primary herb
2. Supporting herbs that strengthen activity of the primary herb
3. Assimilation herbs that augment the assimilation and bioavailability
4. Detoxifying herbs that work on the body's self -repair mechanism so that the primary herb may be used more efficiently

A Combination Index (CI) recognized as the standard measure of combination effect that indicates a greater (CI < 1), lesser (CI > 1) or similar (CI = 1) effect than the expected additive effect. The Bliss Independence model [27, 28, 29, 30] is based on the principle that drug effects are outcomes of probabilistic processes According to this principle, it is proposed that drugs act independently in such a manner that neither of them interferes with the other, but each contributes to a common result. The raw drugs that were combined with *N, cadamba* for this study due to the following properties:

Chemical constituents present in the flowers of *B. monosperma* possesses antihepatotoxic activity [32], antitumorigenic activity [33] anti-oestrogenic activity [34] antihyperglycemic activity [35, 36] antistress activity [37] antiinflammatory and antioxidant activity [38, 39, 40, 41] chemopreventive and anti-cancer properties [42] anticonvulsant activity [43].

The fruit peel of *C. sinensis* is reported to be an abundant source of flavanones and many polymethoxylated flavones, which are very rare in other plants [44]. The citrus fruit has significance as a natural antioxidant as it helps prevent free radicals from damaging the DNA of cells and cause cancer.

H. rosasinensis petals were found to possess abundant phenolic and flavonoid contents and exhibited excellent antioxidant activities, making it a good drug candidate for antioxidant therapy [45].

Leaves of *S. oleracea* are a source of the bioflavonoid quercetin with many other flavonoids which exhibits anti-oxidant, antiproliferative, anti-inflammatory, antihistaminic, CNS depressant, protection against gamma radiation, hepatoprotective properties in addition to its many other benefits.

Loewe additivity has become the basis for the following method used to analyze drug-drug interaction. The isobolo gram analysis evaluates the type of interaction of two drugs, i.e., drug A and drug B, at a given effect level [31]. If the concentrations required to produce a given effect are determined for drug A ($IC_{x, A}$) and drug B ($IC_{x, B}$) and indicated on the x and y axes of a two-coordinate plot, forming the two points ($IC_{x, A}$, 0) and (0, $IC_{x, B}$) then the line connecting these two points is the line of additivity. Then, the concentrations of A and B contained in combination that provide the same effect, denoted as ($C_{A, x}$, $C_{B, x}$), are placed in the same plot. In the case of the four formulations studied, synergy is indicated as ($C_{A, x}$, $C_{B, x}$) is located below the line of additivity.

Combination index (CI) is calculated by the equation:

$$CI = [\, C_{A,x}/ IC_{x,A}] + [\, C_{B,x}/ IC_{x,B}]$$

Results of the study revealed that all the polyherbal formulations as well as the common constituent, *N. cadamba* exhibited synergy in antioxidative ability and better efficacy as compared to the single drug extracts (Table 2). The experiments were carried out in triplicate. The results are given as mean ± standard deviation

Due to the complex nature of phytochemicals present in these plant parts, any single method does not give complete evaluation of the antioxidant activity.

Synergistic effect

The total phenolic content and total flavonoid content were determined for the five tested single plant drugs and the combinations. The combination of *N. cadamba* with *B. monosperma* showed almost fifty percent higher total phenolic contentand almost ten percent higher flavonoid content (Fig. 1) with respect to the expected value for the formulation. With *C. sinensis,* the flavonoid content was lower than expected while total phenolic content was found to be nearly fifty percent higher in its formulation of *N. cadamba*. The synergy in phenolic content was over seventy five percent with *H. rosa-sinensis* though the enhancement of total flavonoid content was just over fifteen percent as compared to the values calculated for the formulations with respect to their individual behaviour., Combination index analysis carried out on the DPPH radical

scavenging activity values revealed that CI values were less than 1 for all four formulations, indicating synergy (Table 3)
The HPTLC studies also revealed that the maximum number of peaks were enhanced in *N. cadamba* + *H. Rosa-sinensis* combination and to the maximum extent amongst all the studied samples (Table5). (Fig 3) Amongst all the tested drugs, the total antioxidant capacity was enhanced to the largest extent in the combination of *N. cadamba* with *H. rosa- sinensis* (Table2). The synergistic effect was lower in formulation with *S. oleracea* for both total phenolics as well as flavonoid contents. Marginal synergy was also observed in Nitric Oxide Radical Scavenging Assay in the *N. cadamba* + *S. oleracea* combination; it was not significant in this test model for the *N. cadamba* combination with the other three plant samples (Fig.1).The values of radical scavenging activity as determined by DPPH method and as determined by Nitric Oxide Radical Scavenging method showed Pearson co-relation (positive) value 0.48754 affirming their linear dependence.
Phenolic content from plant extracts have been found to correlate with radical scavenging activity (Pearson correlation coefficient = 0.46056) as polyphenolics have high redox potentials which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers

4. Tables and figures

Table 1: Phytochemical screening

Sample »	***N. cadamba***	***B .monosperma***	***C. sinensis***	***H. rosa-sinensis***	***S .oleracea***	***N. cadamba+ B .monosperma***	***N. cadamba+ C. sinensis***	***N. cadamba+ H. rosa-sinensis***	***N. cadamba+ S .oleracea***
Alkaloids	+	+	+	+	+	+	+	+	+
Phenolics	+	-	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	-	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+

Table 2: Antioxidant and radical scavenging assay in relation with total phenolic and total flavonoid contents

Name of sample tested	**% RSA by DPPH method**	**TAC by Phospho-molybdenum method (equivalent to ascorbic acid)**	**Nitric Oxide Radical Scavenging Assay**	**Total flavanoid content in quercetin equivalents in mg/g**	**Total phenolic content gallic acid equivalent in mg/g**
Neolamarckia cadamba (N. cad)	17.49± 0.32	16.27± 0.45	39.56 ± 0.23	114.22 ± 0.0111	50.68± 0.1373
Butea monosperma (B. mon)	36.3 ± 0.49	12.04±0.33	49.22± 0.35	369.19±0.0215	114.93±0.21
Citrus sinensis (C. sin)	12.75±0.11	15.12±0.16	39.56±0.09	23.22±0.0198	110.41±0.37
Hibiscus rosa - sinensis(H. ros)	48.42 ±0.50	18.58±0.41	46.57±0.24	383.41±0.0331	137.1±0.12
Spinacea oleracea (S. ole)	29.68±0.21	38.96±0.91	37.66±0.35	77.73±0.0451	19.91±0.21
N. cad+ B. mon	42.78±0.19	19.35±0.66	44.07±0.17	264.93±0.0112	129.19±0.31
N. cad+ C. sin	28.43±0.28	18.19±0.42	39.21±0.35	49.29±0.0156	125.11±0.29
N.cad+ H. ros	55.4±0.17	79.35±0.48	50.97±0.21	373.46±0.0018	145.7±0.25
N. cad+ S. ole	36.5±0.34	47.42±0.51	38.61±0.18	98.59±0.0210	41.18±0.016

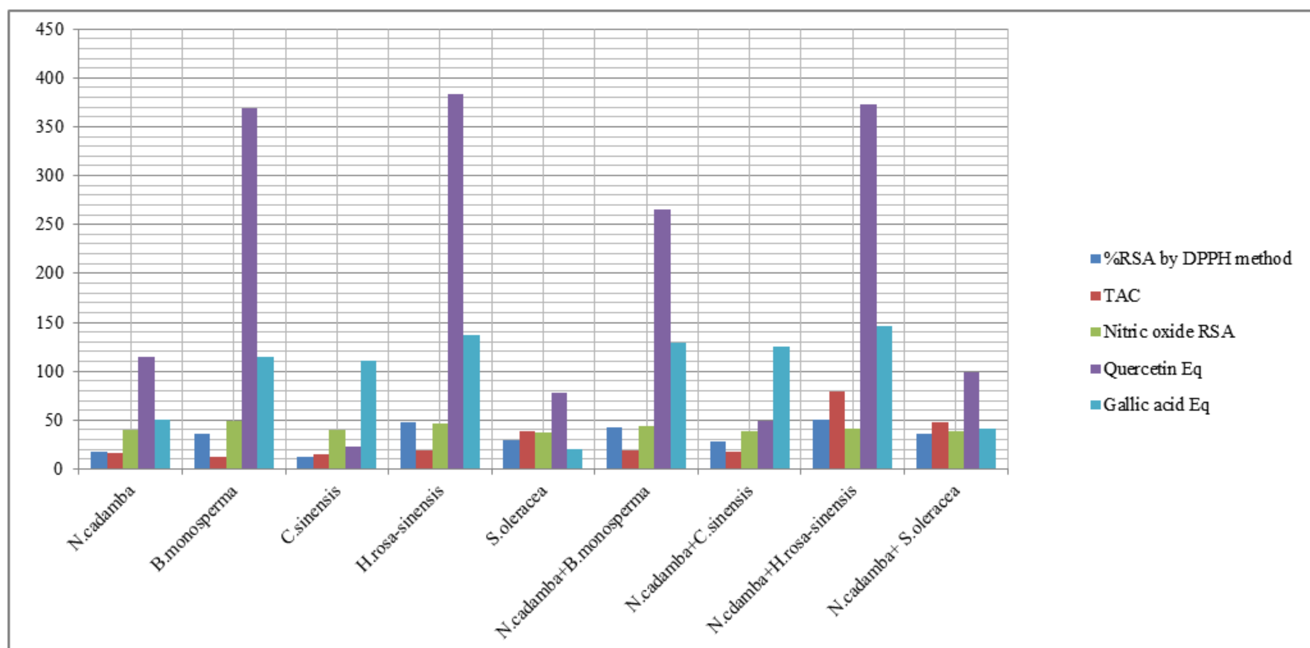

Fig 1: Comparative values of % radical scavenging activity by all three test models method With respect to total flavonoid and total phenolic content

Table 3: Combination index values of the formulations

Sample	$IC_{x, A}$	$IC_{x, B}$	$(C_{A, x}, C_{B, x})$	CI
N. cadamba +B. monosperma	5.718	2.755	2.3375	0.5379
N. cadamba +C. sinensis	5.718	7.843	3.5174	0.3024
N. cadamba +H. rosa-sinensis	5.718	2.0653	1.9610	0.6591
N. cadamba +S. oleracea	5.718	3.3693	2.7397	0.4717

HPTLC studies

Table 4: Solvent systems for HPTLC studies

Name of sample(s) / combination(s) spotted	Solvent system used
(N. cadamba)+(B. monosperma)	Ethylacetate: methanol: water (100:15:5)
(N. cadamba)+(C .sinensis)	Ethylacetate: methanol: water (15:3:2)
(N. cadamba)+ (H. rosa-sinensis)	Toluene: ethyl acetate: acetic acid(9.5:8:5.2)
(N. cadamba)+ (S. oleracea)	Ethanol +n-hexane (1:1)

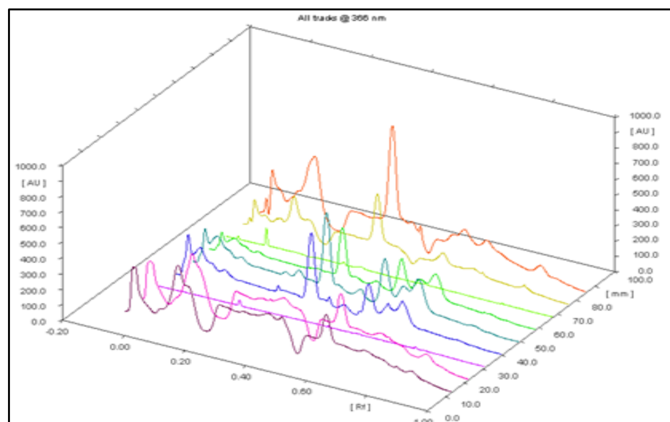

Fig 2: Fingerprinting of 3D display at 366 nm in densitometry detection of *B. monosperma* and its formulation. Track 1 and 2- *B.monosperma,* track 4, 5 and 6 -*N. cadamba*, track 8 and 9: 1:1 formulation of *N. cadamba and B. monosperma*

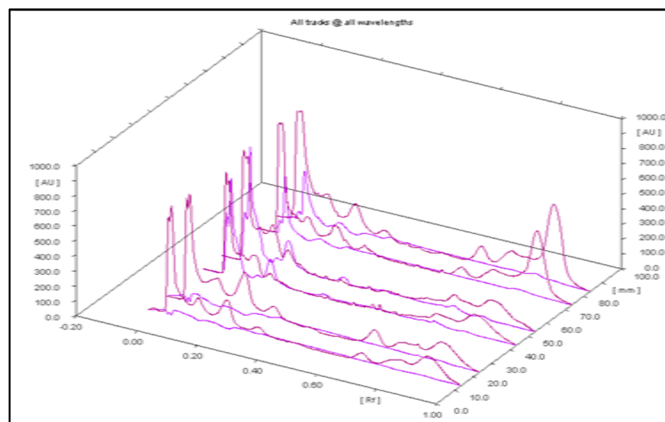

Fig 3: Fingerprinting of 3D display in densitometry detection of *H. rosa-sinensis* and its formulation (MWL) Tracks 1 and 2- *H. rosa-sinensis,* tracks 4 and 5- *N. cadamba,* tracks 7 and 8 - 1:1 formulation of *H. rosa sinensis and N. cadamba*

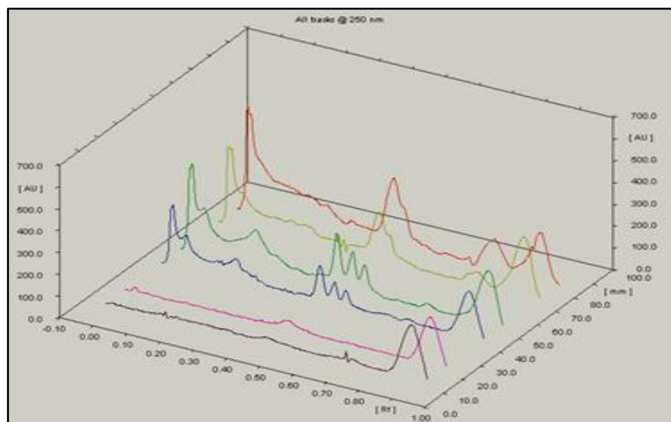

Fig 4: Fingerprinting of 3D display at 254 nm in densitometry detection chromatogram of C. sinensis and its formulation

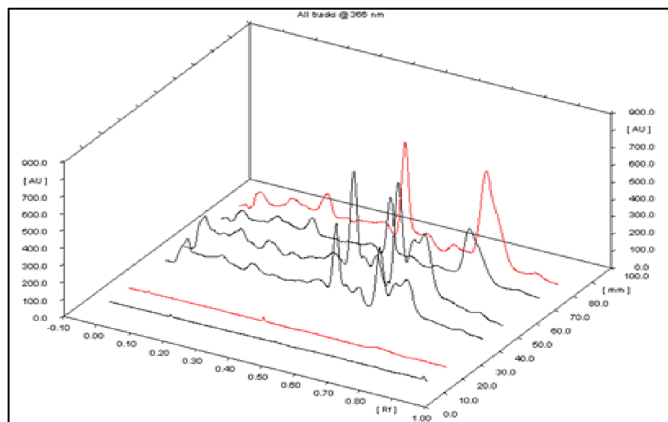

Fig 5: Fingerprinting of 3D display at 366 nm in densitometry detection of *C. sinensis* and its formulation, Tracks 1 and 2- *C. sinensis*, tracks 4 and 5- *N. cadamba,* tracks 7 and 8 - 1:1 formulation of *C. sinensis* and *N. cadamba*

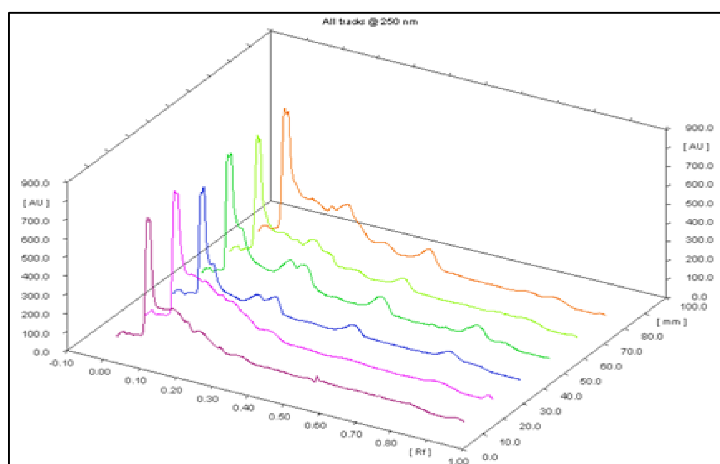

Fig 6: Fingerprinting of 3D display at 254 nm in densitometry detection of *S. oleracea* and its formulation**.** Tracks 1 and 2-*S. oleracea*, tracks 3 and 4- *N. cadamba*, tracks 5 and 6 - 1:1 formulation of *S. oleracea and N. cadamba.*

Table 5: Comparative values of areas under the peaks

Sample	**Peak area enhancement for common R_f values seen for individual and combination of plant materials (at 254 nm) in AU Weight of sample(s) extracted**				**Peak area enhancement for common R_f values seen for individual and combination of plant materials (at 366 nm) in AU Weight of sample(s) extracted**			
		I(1g)	**2(1g)**	**1+2 (0.5g+0.5g)**		**1(1g)**	**2(1g)**	**1+2 (0.5g+0.5g)**
N. cadamba +B. monosperma	------				0.37	14634.6	10942	15588
N. cadamba+ C. sinensis	0.43 0.74	1568.9 346.2	5610.8 287	14499.9 2263.5	0.71	620.4	7856.8	25467.7
N. cadamba +H. rosa-sinensis	0.03 0.64 0.74 0.88	16035.8 3980.6 2562.1 8866.5	16591.4 1950.2 4781.4 10486.5	23082.1 4198.9 6281.5 23590.9	0.16 0.87	1025.9 395.4	1266 512.3	1501.9 1211.8
N. cadamba +S. oleracea	0.21 0.31 0.44	8264.5 3829.9 645.1	7082.7 1363.4 3094.2	14366.8 4697.9 3888.7	0.06	7088.8	12272.6	16771.2

Sample 1: *B. monosperma/ C. sinensis/ H. rosa-sinensis/ S. oleracea* Sample 2: *N. cadamba*

5. Conclusion

The results of antioxidant evaluation based on the two models (DPPH &Nitric oxide radical scavenging assay) used in this study revealed that maximum synergy in antioxidant activity of the formulation of *N. cadamba* and *H. rosa- sinensis* as compared to single plants. This observation was supported by the increased quantity of total phenolics in the formulations as compared to the single plants. The correlation analysis between

the values of DPPH and Nitric oxide radical scavenging assay indicates the viability of the two models for evaluating antioxidants from medicinal plants. HPTLC studies revealed that the peak area for some R_f values validates the increase in the corresponding active constituent in the formulations as compared to the single plants. This finding therefore reinforces the potentials as important source of natural antioxidants which may provide protection against free radicals induced damage to biomolecules. The polyherbal formulations may offer safer alternatives for treatment of cancer, Parkinson's, Alzheimer's disease and pollution induced health problems [46, 47].The significant antioxidant activity of the formulations may render them suitable for use as radioprotective agents [48].

6. References

1. M Iqbal Choudhary M Mosihuzzaman. Protocol on safety, efficacy, standardization and documentation of herbal medicines. Pure and Applied Chemistry. 2008; 80(10):2195-2230.
2. Oluyemisi Folashade, Egharevba, Henry Omoregie and Ahmadu, Peter Ochogu Kunle. Standardization of herbal medicines-A review. International Journal of Biodiversity and Conservation. 2012; 4(3):101-112.
3. Patil A. Phaatak A, Chandra N, Lobo V. Free radicals, antioxidants and functional foods- Impact on human health. Pharmacogn Rev, 2010; 4:118-126.
4. Grete B Helle LM. Spices as Antioxidants. Trends in Food Science and Technology, 1995; 6:271-277.
5. Bylund DB, Toews ML. Pharmacologic Principles for Combination Therapy. Proc Am Thorac Soc, 2005; 2(4): 282-9.
6. Reid JL, Pharmacokinetic, pharmacodynamic aspects of the choice of components of combination Therapy. J Hum Hypertense. 1995; 9:19-23.
7. Koike K, Jia ZH, Achari B, Banerjee S, Nikaido T. Sahu NP. Structure of two novel isomeric triterpene saponins from Anthocephalus cadamba. Magnetic Resonance in Chemistry. 1999; 37:737-742.
8. Gupta SK, Vasisht K, Keene AT, Phillipson JD. Handa SS. Quinoline alkaloids from Anthocephalus chinensis. Planta Medica. 1984; 50:358.
9. Wei H, Nagao S, Mahmud T, Hori K, Kobayashi M, Uji T, *et al.* Kitagawa I. Characterization of 3′-O-caffeoyl sweroside, a new secoiridoid glucoside, and kelampayosides A and B, two new phenolic apioglucosides, from the bark of Anthocephalus chinensis. Chemical and Pharmaceutical Bulletin, 1996; 44:1162-1167.
10. Chapple CL, Brown RT, Anthocephalus alkaloids: cadamine and isocadamine. Tetrahedron Letter. 1976; 19:629-630.
11. Jyothi Pinnapareddy, Sravan Kumar Bussa. Antidiabetic activity of stem bark of Neolamarckia cadamba in alloxan induced diabetic rats. International Journal of Pharmacy and Technology. 2010; 2(2):314-324.
12. Kumar GS, Jayaveera KN, Kishore DVK, Ashok-Kumar CK, Dhanpal R. *et al.* Antimicrobial, wound healing and antioxidant activities of Anthocephalus cadamba. Afr J Tradit Complement Altern Med. 2007; 4:481-487.
13. Darji Vinay C, Bariya Aditi H, Patel Kaushik R, Sonpal Rakshit N Patel, Divyakant A. Evaluation of antifungal activity of Neolamarckia cadamba (Roxb) Bosser leaf and bark extract. International Research Journal of Pharmacy, 2011; 2(5):192-193.
14. Gouri Kumar Dash, Suman Acharyya Sumanta Mondal. Analgesic, anti-inflammatory and antipyretic studies of Neolamarckia cadamba barks. Journal of Pharmacy Research, 2009; 2(6):1133-1136.
15. Niharika Tomar, Ashish Kumar Gupta Deepak Ganjewala. Phytochemical composition and antioxidant properties of methanol extracts of leaves and fruits of Neolamarckia cadamba (Roxb).Journal of Biologically Active Products from Nature, 2013; 3(4):232-240.
16. Prabhakar Sharma, Ramchandra Gupta, Sudhakar Sharma Sunil Roshan. Butea monosperma: A traditional medicinal plant:-An Overview. Pharmatutor.
17. Bhaskara R, Krishnaswamy H Murti P, in Proceedings-Indian Academy of Sciences, Section A (12A). 1940; 472-476.
18. Patil PH, Priti S. Tidke and Prasad G. Jamkhande.In vitro antioxidant activity of Butea monosperma flower fractions. International journal of Drug Development and Research, 2013; 5(3):245-255.
19. Galvano F, Mistretta A, Marventano S, Nolfo F, Calabrese G.and Grosso G. Red orange: experimental models and epidemiological evidence of its benefits on human health.Oxid Med Cell Longev, 2013.
20. Lopez CNP, Magalong AL. Phytochemical contents and antimicrobial properties of floral extracts from different gumamela cultivars. AGRIS, 2007.
21. Abdul Aziz MWH, Roslida AH, Zakaria ZA, Zuraini A, Hakim Raduan SZ MN. Antiinflammatory effects of Hibiscus Rosa sinensis and Hibiscus rosa- sinensis Var Alba ethanol extract. International Journal of Pharmacy and Pharmaceutical Sciences, 2013; 5(4):754-762.
22. Sowmia C, Dhanya KP, Joona K, Divya M J. Screening of antioxidant, anticancer and phytochemicals in methanolic extract of Hibiscus rosa-sinensis leaf extracts. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2013; 4(2):1308-1316.
23. Anwar F. and Sultana B. Flavonols (kampeferol, quercetin, myricetin) contents of selected fruits. Food Chem, 2008; 108:879-884.
24. Bunea A, Socaciu C, Bobis O, Neacsu M, Verne R *et al.* Andjelkovic M. Total and individual carotenoids and phenolic acids content in fresh, refrigerated and processed spinach(Spinacia oleracea). Food Chem, 2008; 108:649-56.
25. Marisol Castaner, Francisco A, Tomas Barberan and Federico Ferreras. Acylated flavonol glycosides from spinach leaves (Spinacia oleracea). Phytochemistry. 1997; 45(8):1701-1705.
26. Shete Rajkumar, Virbhadrappa, Otari Kishor, Vasant Gaikwad, Priyanka Subhash. Spinacia oleracea Linn:A pharmacognostic and pharmacological overview. International Journal of Research in Ayurveda and Pharmacy, 2010; 1(1):78-84.
27. BlissCI. The toxicity of poisons applied jointly. Ann Appl Biol. 1939; 26:585-615.
28. Berenbaum M. What is synergy? Pharmacol Rev. 1989; 41:93-141.
29. Bravo G, Parsons JC, Greco WR. The search for synergy: a critical review from a response surface perspective. Pharmacol Rev. 1995; 47:331-385.

30. Geary N. Understanding synergy. Am J Physiol Metab. 2013; 304:237-253.
31. Thomas R Fraser. The antagonism between the actions of active substances. The British Medical Journal, 1872; 485-487.
32. Shukla S. *et al*. Sharma N. Hepatoprotective potential of aqueous extract of Butea monosperma against CCl (4) induced damage in rats. Exp Toxicol Pathol. 2011; 63(7-8): 671-6.
33. Fatima G, Saxena AK, Chandan BK, Jaggi BS, Gupta BD, *et al*. Mathan G. Chemoprevention with aqueous extract of Butea monosperma flowers results in normalization of nuclear morphometry and inhibition of a proliferation marker in liver tumors. Phytother Res. 2011; 25(3):324-8.
34. Raina MK, Sharma M, Satyawati GV. Medicinal Plants of India, 1st ed., Indian Council of Medical Research, 1976, 1.
35. Sanjay Kasture, Abhay Kumar Singhai *et al.* Rahul Somani. Antidiabetic potential of Butea monosperma in rats. Fitoterapia. 2006; 77(2):86-90.
36. Garg V. *et al* Sharmna N. Antihyperglycemic and antioxidative potential of hydroalcoholic extract of Butea monosperma Lam flowers in alloxan-induced diabetic mice. Indian J Exp Biol, 2009; 47(7):571-6.
37. Chintawar SD, Logade NA, Somani RS, Kasture VS, Kasture SB, Bhatwadekar AD. *Et al.* Antistress activity of Butea monosperma flowers. Indian Journal of Pharmacology. 1999; 31(2):153-155.
38. Michel T, Destandau E, Reddy M, André P, Elfakir C, *et al.* Krolikiewicz-Renimel I. Antiinflammatory and antioxidant activity: Antioxidant, anti-inflammatory and matrix metalloproteinases inhibitory activities. J Ethnopharmacol. 2013; 148(2):537-43.
39. Desai SK. *et al* Shahavi VM. Anti-inflammatory activity of Butea monosperma flowers. Fitoterapia, 2008; 79(2):82-85.
40. Kumar V. *et al*. Sehrawat A. Butein imparts free radical scavenging, anti-oxidative and proapoptotic properties in the flower extracts of Butea monosperma. Biocell. 2012; 36(2):63-71.
41. Mishra SH. *et al.* Lavhale MS. Evaluation of free radical scavenging activity of Butea monosperma Lam, Indian J Exp Biol, 2007; 45(4):376-84.
42. Shukla SK, Kumar V. *et al.* Choedon T. Chemopreventive and anti-cancer properties of the aqueous extract of flowers of Butea monosperma. J Ethnopharmacol, 2010; 129(2):208-213.
43. Kasture SB, Chopde CT. *et al.* Kasture VS. Anticonvulsive activity of Butea monosperma flowers in laboratory animals. Pharmacol Biochem Behav, 2002; 72(4):965-72.
44. Salim-ur-Rehman ZMM, Iqbal, Anjum FM, JI. Sultan Ahmad. Genetic vari ability to essential oil composition in fourcitrus fruit species. Pak. J. Bot, 2006; 38(2):319-324.
45. Vijayalakshmi Melanathuru, Deecaraman Munuswamy, Sumathy Rengarajan. Comparative studies of antioxidant studies of antioxidant activity from the petals of selected Indian medicinal plants. International Journal of Pharmacy and Pharmaceutical Sciences, 2015; 7(6).
46. D Harman. Ageing, A theory based on free radical and radiation Chemistry. J Gerontol, 1956; 11: 298-300.
47. JMC Gutteridge, Halliwell B. Free Radicals in Biology and Medicine, Oxford University Press, 2015.
48. Scarpelli MS, Cotelli *et al*. Filosto M. The role of mitochondria in neurodegenerative diseases. J. Neurol. 2011; 258(10):1763-74.