ABSTRACT

Neolamarckia cadamba / Anthocephalus cadamba (Roxb.) Miq. Syn A. chinensis (Lamk) A. Rich (Rubiaceae) (A.cadamba) has been widely used in various ayurvedic formulations in the form of churna (nygrodhadi kvatha churna herbal formulation) and oil (grahanimihira taila). It is ethnomedicinally widely used in the form of a paste by tribes in the western Ghats for treating skin diseases and wound healing. The methanol extract of N.cadamba is rich in phenolic and flavonoid contents and exhibits marked antioxidant potential by inhibiting lipid peroxidation and increase in the superoxide dismutase (SOD) and catalase activity. A.indica is reported to exhibit antioxidant activity, antidiabetic activity, antimicrobial activity, antifungal activity, and anticancer activity in literature. The methanolic extracts of N.cadamba, A.indica and their 1:1 formulation was tested for free radical scavenging activity by three test models, viz. DPPH method, Nitric oxide scavenging assay and phosphomolybdenum method. The formulation produced enhanced activity compared to the individual drugs, viz. was superaddITIVE i.e. synergistic; demonstrating action that is above what is expected from their individual potencies and efficacies. HPTLC fingerprinting done for the formulation revealed enhancement of area under the peak for Rf value corresponding to the phytochemical(s) responsible for synergy.

Keywords: Neolamarckia cadamba, synergy, antioxidant, free radical

INTRODUCTION

The World Health Organization defines traditional medicine as “the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness”. India has the unique distinction of having six recognized systems of traditional medicine, viz. Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homoeopathy. Of these, Ayurveda is the oldest and the most popular to this day. Under the traditional system of medicine, only medicinal plants are used for the preparation of herbal drugs whereas alternative medicines are derived from herbs, minerals, and organic matter. Use of plants as a source of medicine has been an ancient practice and is an important component of the health care system in India. India has a diverse agro-climatic profile inclusive of a vast resource of botanical ingredients and plant materials. Plant material and derivatives are used in food and nutrition, medicine, cosmetics, as raw materials for beverages, as insect repellents and several other purposes. It is reported that forty three percent of the total flowering plants prevalent in India possess medicinal uses while approximately 25 percent of the available prescription drugs are derived from medicinal plants.
Rasayana Chikitsa helps to maintain human health and devises methods to rejuvenate life treating the organism holistically. There is an ever increasing interest in the search and use of plant based formulations, leading to a rapidly expanding market for Ayurvedic, neutraceutical and polyherbal formulations. The trade in plant products has increased following their increased use in the food and cosmetic industry. Therapeutic potential of medicinal plants as a source of natural antioxidants, anticancer and antimicrobial agents has been well recognized all over the world.

SYNERGY AND ITS MECHANISM

A plant is essentially a biosynthetic laboratory for a multitude of biomolecules, exerting physiological and therapeutic effects. The pharmacological efficacy of plants arises from the presence of biologically important compounds that play a vital role in the mechanisms of metabolic reactions. These compounds are generally secondary plant metabolites, like alkaloids, tannins, volatile oils, steroids, etc. Among these, phenolics and flavonoids are recognized for their multiple biological effects including antioxidants, antiinflammatory, anti-microbial, anti-carcinogenic properties, etc. (Rice-Evans C. A. 1996) (Cowan MM 1999)

In some cases, the active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects; but in certain ratios, multiple herb combinations give a better therapeutic effect and reduce the toxicity. (Srivastava S 2013) (G. S. Subramani Parasuraman 2014). Synergy implies the creation of a whole that is more than the simple sum of its parts. The term synergy comes from the Attic Greek word synergia ( confer Koine Greek: synergieia) from synergos, meaning “working together”. According to Western Medicine, when the effects of two chemicals on an organism are greater than the effect of each chemical individually or the sum of the individual effects, synergy is said to have resulted from the combination. The concept of polyherbalism is peculiar to Ayurveda, although it is difficult to explain in terms of modern parameters. Ayurveda furnishes the fundamental aspects for drug formulation. The herbs are selected according to the disease; other herbs may be used to prevent the side effects arising from the use of the chief herb(s).

Typical formulations for fever include herbs like parpatadi quath, kirat-tikatadi quath. The Polyherbal formulations have been in use for centuries. (RastogiRP n.d.) Drug formulation in Ayurveda can involve the use of a single drug or use of more than one drug, known as polypharmacy or polyherbalism; i.e., the combining of several medicinal herbs to achieve extra therapeutic effectiveness. The active phytochemical constituents of individual plants usually are present in minute amounts and almost always, in insufficient amounts to achieve the desirable therapeutic effects. Studies have revealed that plants of varying potency when combined may theoretically produce a greater result, as compared to individual use of the plant and also the sum of their individual effects. This phenomenon of positive herb-herb interaction is known as synergism. In traditional medicine whole plants or mixtures of plants are used rather than isolated compounds. There is evidence that crude plant extracts of often have greater in vitro or/and in vivo antiplasmodial activity than isolated constituents at an equivalent dose. Among the mechanisms proposed for synergism ; (i.e. pharmacodynamics and pharmacokinetic, (SpinellaM 2002) the former studies the synergistic effect when active constituents with similar therapeutic activity are targeted to a similar receptor or physiological system, while the latter proposes it on the ability of a herb to facilitate the absorption, distribution, metabolism and elimination of the other herbs. A combination of herbals may simultaneously act on multiple targets to eradicate the disease causing complicated factors and offer thorough relief. (ChorgadeMS 2007)

MATERIALS AND METHODS

In this study, fresh bark of Neolamarckia cadamba, collected from Somaiya Vidyavihar Campus and seeds of Azadirachta indica collected from vegetation in the locality were composed into a 1:1 (w/w ratio) novel formulation. Both the raw herbs were authenticated at Blatter Herbarium, St Xaviers’ College, Mumbai. The single plant materials and their formulation were separately
extracted using methanol by Soxhlet extractor. These were standardized according to the AYUSH protocol and HPTLC fingerprinting was also done. For testing antimicrobial and antifungal activities, the dried, ground, sieved and weighed quantity of the plant material was extracted at room temperature in ethanol for ten days. The soaked material was stirred every 18 h with a sterile glass rod. The final extracts were passed through Whatman filter paper No 1, concentrated under vacuum on a rotary evaporator at about 40°Celsius.

The materials were authentication and standardization of raw materials was carried out as per the standard protocol published by the Ministry of AYUSH, Government of India.

RESULTS AND DISCUSSIONS

Preliminary phytochemical studies revealed the presence of alkaloids, flavonoids, phenolics, saponins, steroids and terpenoids in the individual drugs as well as the formulation. HPTLC fingerprinting was done using Chloroform: ethyl acetate: formic acid as the mobile phase. Detection at 254 nm and 366 nm showed modifications of peak areas for two Rf values at 254 nm.

<table>
<thead>
<tr>
<th>254 nm</th>
<th>366 nm</th>
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</thead>
<tbody>
<tr>
<td>Rf value</td>
<td>AI Peak Area in AU</td>
</tr>
<tr>
<td>0.06</td>
<td>7088.8</td>
</tr>
<tr>
<td>Diminished</td>
<td>0.93</td>
</tr>
</tbody>
</table>

(Notations – AI- Azadirachta indica, NC- Neolamarckia cadamba, Combination –AI+NC )

3D Densitogram of the methanolic extract of the Formulation and its constituents at 254nm
Lane 1 and 2- A. indic, Lane 4and 5- N. cadamba , Lane 7 and 8- Formulation

Antioxidant activity was evaluated by three methods, while the Total Phenolic content was studied by Folin –Ciocalteau Method and Total Flavonoid Content was found by Aluminium Chloride colorimetric method.
The total antioxidant capacity and % inhibition has enhanced in the case of the Formulation as compared to individual drugs by all three test models. Subsequently, there is an increase in the antioxidant activity due to a synergistic effect with increase in phenolic and flavonoid contents in case of the individual constituents as well as the Formulation.

Pearson’s correlation coefficient values for phytochemical content and antioxidant activity

<table>
<thead>
<tr>
<th></th>
<th>DPPH method</th>
<th>NO RSA method</th>
<th>TAC</th>
<th>Total phenolic content</th>
<th>Total flavonoid content</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH method</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO RSA method</td>
<td>0.322665</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TAC</td>
<td>0.974142</td>
<td>0.10047</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total phenolic content</td>
<td>0.671133</td>
<td>0.918234</td>
<td>0.4863</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total flavonoid content</td>
<td>0.721073</td>
<td>0.888465</td>
<td>0.545887</td>
<td>0.997578</td>
<td>1</td>
</tr>
</tbody>
</table>

The total phenolic concentration of the seed oil of A.indica showed a positive correlation with antioxidant capacity. A reported combination of Vernonia amygdalina and A. indica showed a positive synergism in antioxidant activity, due to a boost in the flavonoid content of the extracts. (Atangwo I.J. 2012) The Formulation showed significant positive correlation (0.9976) between the total phenolic and the flavonoid contents. Also, the positive correlation between total phenolic as well as total flavonoid contents with the antioxidant activity measured using three different methods confirmed the enhancement in the antioxidant activity due to the synergistic effect of the two constituents.

Combination index values

Combination Index is recognized as one of the most practical and demonstrative methods to evaluate combination effect and prove synergy experimentally. It is generally applicable for two drug combinations (Xian Zhou 2016). A Combination Index (CI) recognized as the standard measure of combination effect that indicates a greater (CI < 1) (synergy), lesser (CI > 1) or similar (CI = 1) effect than the expected additive effect.

<table>
<thead>
<tr>
<th></th>
<th>CI value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH Test</td>
<td>0.3965</td>
</tr>
<tr>
<td>NO Scavenging Assay</td>
<td>0.779</td>
</tr>
<tr>
<td>TAC Assay</td>
<td>0.791</td>
</tr>
</tbody>
</table>
The synergistic effect of the two drugs was further verified using combination index method as reported by Chou. (Chou 2010) The combination index observed by all the three methods is less than one. (Tables 3.1e, f and g) Hence the combination effect is more pronounced than the simple additive effect in the case of Formulation 1. The low value of CI indicates that the relatively lower dose of the formulation will be effective than either of the single drugs.

**Anticancer activity**

The Formulation was tested for anticancer activity along with its constituent raw drugs viz. *N.cadamba* and *A.indica* by in-vitro SRB (Sulphorhodamine –B) assay method. The average of three experiments revealed that the Formulation at drug concentrations of 40 and 80 μg/mL shows synergy by decreasing the % control growth of human breast cancer cell line MCF-7 from 90.2 and 99.5 for single plant drug extract to 83.3 and 86.9 respectively, in its formulation. In case of human prostate cancer cell line PC-3 the effect of synergistic action is observed for 80 μg/mL concentration; wherein the % control growth decreased from 91.7 to 88.5, while in Mouse Skin melanoma it decreased from 82.2 to 79 at 40 μg/mL for the Formulation as against the single plant drug extract. The % control growth of human hepatoma cell line Hep-G2 was decreased from 152.2 to 148.7.
CONCLUSION

Among the many pharmacological activities for which herbal medicines are used, antioxidant activity is fast gaining importance. Toxic substances, pollution, auto exhaust, smoke, additives etc. accelerate the formation of free radicals in biological systems. These cause more than 60 human diseases including cancer, heart disease and the acceleration of the aging process. As a result, worldwide, tremendous research interest has been generated in the quest for natural antioxidants. This finding therefore reinforces their potential as important sources which may provide protection against free radicals induced damage to biomolecules. The polyherbal formulations may offer safer natural product remedies as alternatives for treatment of cancer, Parkinson’s, Alzheimer’s disease and pollution induced health problems

Thus, synergy of antioxidant activity is coupled with synergy of anticancer activities in the studied formulation as mentioned. This observation is of much significance in the current trend of targeted combination drug therapy leading to lesser side effects of any of the drugs in isolation.

REFERENCES