A Comparative Study of Anti-inflammatory Activity of *Tecoma stans, Acalypha indica* and *Abutilon indicum* plant leaf extract

Running title: Anti-inflammatory activity of *Acalypha indica,* A. indicum and *Tecoma stans*
Type of study: Original research study

ABSTRACT:

Introduction: Inflammation is said to be the response of the body to an injury. It is a body defence reaction to reduce or eliminate the spread of injurious agents. It is essential that steps should be taken to introduce new medicinal plants and to develop cheaper, effective and safe analgesic and anti-inflammatory drugs. The main aim of this study is to assess the potential anti-inflammatory activity of *Tecoma stans*, *Acalypha indica* and *Abutilon indicum* plant is being studied.

Materials and Methods: Protease inhibition assay was done by Bovine serum albumin was added to plant samples with increase in concentrations as per the standard methods. In this study, Aspirin was used as a standard anti-inflammatory drug.The data were analyzed statistically by a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to see the statistical significance among the groups. The results with $p<0.05$ level were considered to be statistically significant.

Results: In this study, it was observed that the plant leaf extract of *Tecoma stans*, *Acalypha indica* and *Abutilon indicus* contain anti-inflammatory activity. The protein denaturation inhibitory activity of leaf extract of *Tecoma stans*, *Acalypha indica* and *Abutilon indicum,* plant extract was represented graphically. *Tecoma stans*, was observed to contain the anti inflammatory activity.

Conclusion: This study revealed that *Tecoma stans*, *Acalypha indica* and *Abutilon indicum* are important medicinal plants with diverse pharmacological spectrum and contain anti-inflammatory properties. Hence, this research has been taken to collect and compile the

pharmacological uses of these plant extracts which will be useful to the society to venture into a field of alternative systems of medicine.

Keywords: Inflammation, Pharmacological uses, medicinal plants, plant extracts, Innovative techniques

INTRODUCTION:

Pain and inflammation is considered to be one of the most common and major health problems. Excess of the inflammatory responses has got damaging effects such as septic shock that could lead to multiple organ dysfunction syndrome and even death. Inflammation is said to be the response of the body to an injury (1). It is a body defence reaction to reduce or eliminate the spread of injurious agents. There are several components of the reaction that contribute to the associated symptoms and tissue injury. Inflammatory response involves a complex array of enzyme activation, mediator release fluid extravasations, cell migrations, tissue breakdown and repair which are aimed at host defence and usually activated in most disease conditions. Components like edema formation, leukocyte infiltration and granuloma formation represent such inflammations (1,2). The drugs that are used at present for the management of pain and inflammatory situations are either narcotic or not narcotic, steroidal or non-steroidal anti-inflammatory drugs . They are known for their toxic and lethal effects (3). Most of the drugs usually undergo some gastrointestinal damage due to the inhibition of the protective cyto oxygenase enzyme in gastric mucosa. On the other hand herbal and natural medicines have good absorption, less toxicity and also easy availability. They have also been used since ancient times (1,2,4).

Tecoma stans, is a species belonging to the trumpet vine family, Bignoniaceae that is native to America. It possesses a lot of other synonyms such as *Bignonia stans L, Kuntze, Stenolobium stans (L).* It is also commonly called Yellow trumpet bush, Yellow bells, Yellow

elder, Ginger-Thomas and Esperanza. It is a flowering perennial shrub or a small tree that is 5m to 7.6m in height. The bark is pale brown to grey and rough (5). The fruits of this plant are narrow and slightly flattened to provide capsules, up to 20 cm long, containing many winged seeds; greenish yellow, young, pale brown on ripening and would remain on its tree in clusters for several months. Its leaves, bark and roots contain many biologically active chemicals and extract from their tissues that's been used in traditional folk medicines (6). *Tecoma stans* were also investigated for antifungal effects in its roots. Standardisation of a plant is the first requirement for its use in herbal medicine.

Acalypha indica, known as 'kuppaimaini' in Tamil, belongs to the family Euphorbiaceae. It is common in many parts of Asia. It grows in common farmland roadside wastelands. Leaves, roots, stalks and flowers of this plant have various uses. These plants are used as laxatives and diuretics. The major phytochemical constituents are alkaloids, acalypus and aclyphine (5,7). *Abutilon indicum* is found in outer Himalayas tracts of Jammu and Kashmir to Bhutan up to an altitude of 15000 m and extending through the whole of northern and central India (8). They are beneficial in treating gout tuberculosis and bleeding disorders as well. Many plant extracts from nature are used to treat various diseases. It is important to promote such methods and give more preference to them.

Our team has extensive knowledge and research experience that has translate into high quality publications (9), (10), (11), (12), (13), (14), (15), (16), (17), (18), (19), (20), (21), (22), (23), (24), (25), (26), (27), (28).

The aim of this study is to compare the anti-inflammatory activity of *Tecoma stans*, *Acalypha indica* and *Abutilon indicum* plant leaf extracts.

MATERIALS AND METHOD:

Chemicals:-

All chemicals and reagents used for this research work were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA

Collection of plant material

The *Tecoma stans*, *Acalypha indica* and *Abutilon indicum* leaves were collected from Chennai District, Tamil Nadu, India. The species were identified and authenticated at the Department of Centre for Advanced Study in Botany, University of Madras, Chennai, India. The bark, leaves and flower parts of the plant were shade-dried, cut into small pieces and coarsely powdered. The coarse powder was used for extraction with ethanol.

Preparation of plant extracts

1kg of dry powders from leaves from both plants were taken in individual aspirator bottles; 3 liters of ethanol was used and the mixture was shaken occasionally for 72 hours. Then the extract was filtered. This procedure was repeated three times and all extracts were decanted and pooled. The extracts were filtered before drying using whatman filter paper no 2 on a Buchner funnel and the solvent was removed by vacuum distillation in a rotary evaporator at 40˚C; the extracts were placed in pre-weighed flasks before drying .

Assessment of *in vitro* anti-inflammatory activity by plant extract:-

Protein denaturation inhibition assay - Inhibition of albumin denaturation

The anti-inflammatory activity of the plant extract was studied by the inhibition of albumin denaturation technique which was studied according to the methods of Mizushima and Kobayashi, 1968 and Sakat et al (2010) followed with minor modifications. The reaction mixture consisted of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using a small amount of 1N HCl. The plant extract collected with increased concentration (100 to 500 µg/ml) were incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, after cooling the turbidity of the sample was measured at 660 nm. (UVVisible Spectrophotometer Model 371, Elico India Ltd) The experiment was performed in triplicate. In this study, Aspirin was used as a standard anti-inflammatory drug.

Calculation:- $\text{Inhibition\%} = 100 - ((A1 - A2)/A0) * 100)$

Statistical analysis:-

The data were analysed statistically using one way analysis of variance (ONE-WAY ANOVA). Duncan Multiple range test was used to analyze the statistical significance between groups. The levels of significance were considered at the levels of $p<0.05$.

RESULT:

In this study, the standard drug used is Aspirin. In **Figure 1**, the yellow bar represents the standard (aspirin) and the green bar represents the leaf extract of *Tecoma stans*. In **Figure 2,** the purple bar represents the standard (aspirin) and the green bar represents the seed extract of *Acalypha indica.* In **Figure 3**, the purple bar represents the standard (aspirin) and the green bar represents the seed extract of *Abutilon indicum.* It can be observed that as the concentration of standard (aspirin) increases from 100 micro g/ml to 500 micro g/ml in each of the graphs obtained, the concentration of the leaf extract also increases.

As a part of the investigation on anti-inflammatory activity conducted by Swarna S K Et al 2019 (29), the ability of different concentrations of *Tecoma stans* ethanolic extracts showed differential inhibitory activity. The leaf, bark and flower extracts were compared to determine which part is most effective. Leaf extract showed anti inflammatory activity at 100, 200, 300, 400 & 500 micro g/ml and 100% of concentration and % inhibition respectively while bark extract showed an anti inflammatory activity at 100, 200, 300, 400 & 500 micro g/ml and 85% of concentration and % inhibition respectively. The flower part of the plant showed an anti-inflammatory activity at 100, 200, 300, 400 & 500 micro g/ml and 100% concentration and % inhibition respectively. They were compared with the commercially available synthetic anti inflammatory drug Ibuprofen, used as standard. Among the different parts of the plant extracts used, the leaf and flower were found to be more effective than bark.

There is a 10% difference in inhibition in *Tecoma stans* compared to the standard Aspirin drug at 500 micro g/ml. Hence, the minimum % inhibition differences between *Tecoma stans* extracts and Aspirin was observed at 500 micro g/ml (figure 1). There is a 10% difference in inhibition in *Acalypha indica* compared to the standard Aspirin drug at 500 micro g/ml. Hence, the minimum % inhibition differences between *Acalypha indica* extracts and Aspirin was observed at 500 micro g/ml (figure 2). There is a 10% difference in inhibition in *Abutilon indicum* compared to the standard Aspirin drug at 500 micro g/ml. Hence, the minimum % inhibition differences between *Abutilon indicum* extracts and Aspirin was observed at 500 micro g/ml. Protein denaturation is considered to be the most common cause of prolonged inflammation. Hence, inhibition of such denaturation can have a clinically favorable effect on inflammation (figure 3).

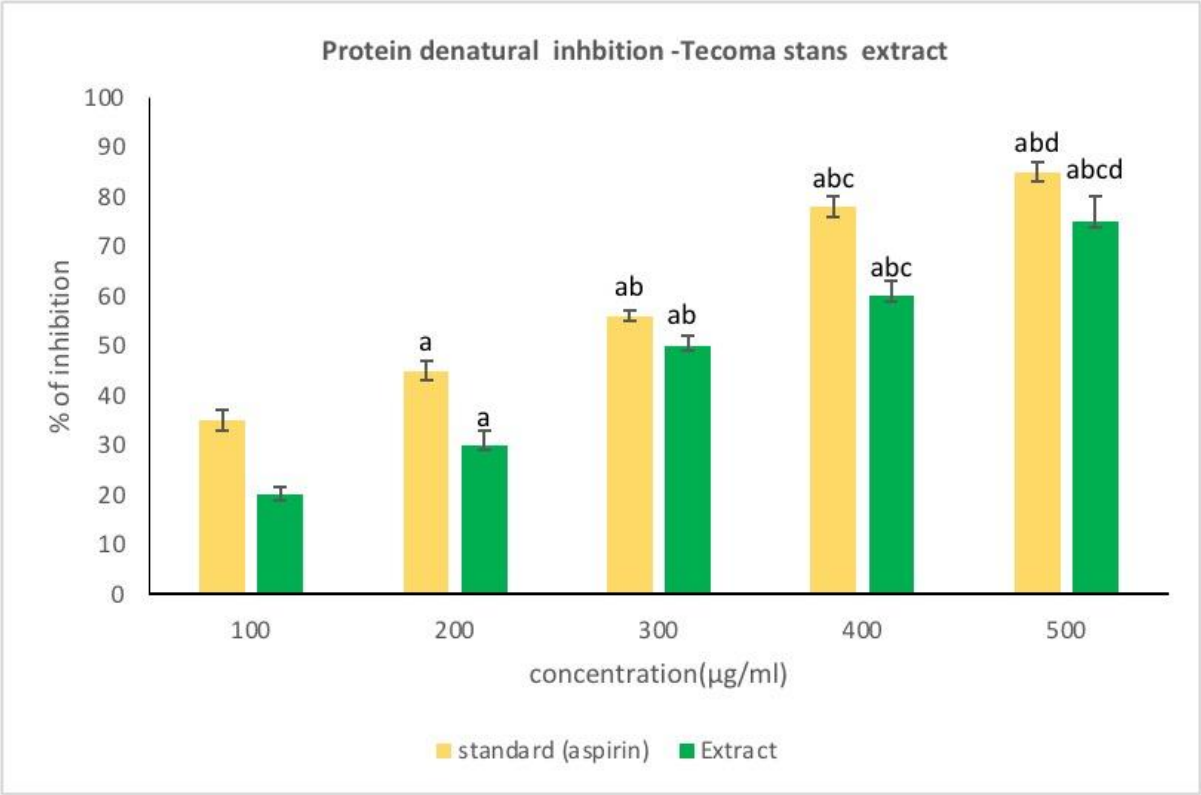

Figure 1 :- Bar graph depicts the Protein denaturation inhibitory activity of leaf extract of *Tecoma stans.* Each bar represents the mean ± SD of 6 observations. The **x-axis** represents the concentration (micro g/ml) of the plant extract and the **y-axis** represents the % of inhibition of the plant extract. The yellow bar represents the standard (aspirin) and the green bar represents the extract. Significance at the levels of $P < 0.05$. a-compared with 100 µg; b-compared with 200 µg; c-compared with 300 µg.; d-compared with 400µg.

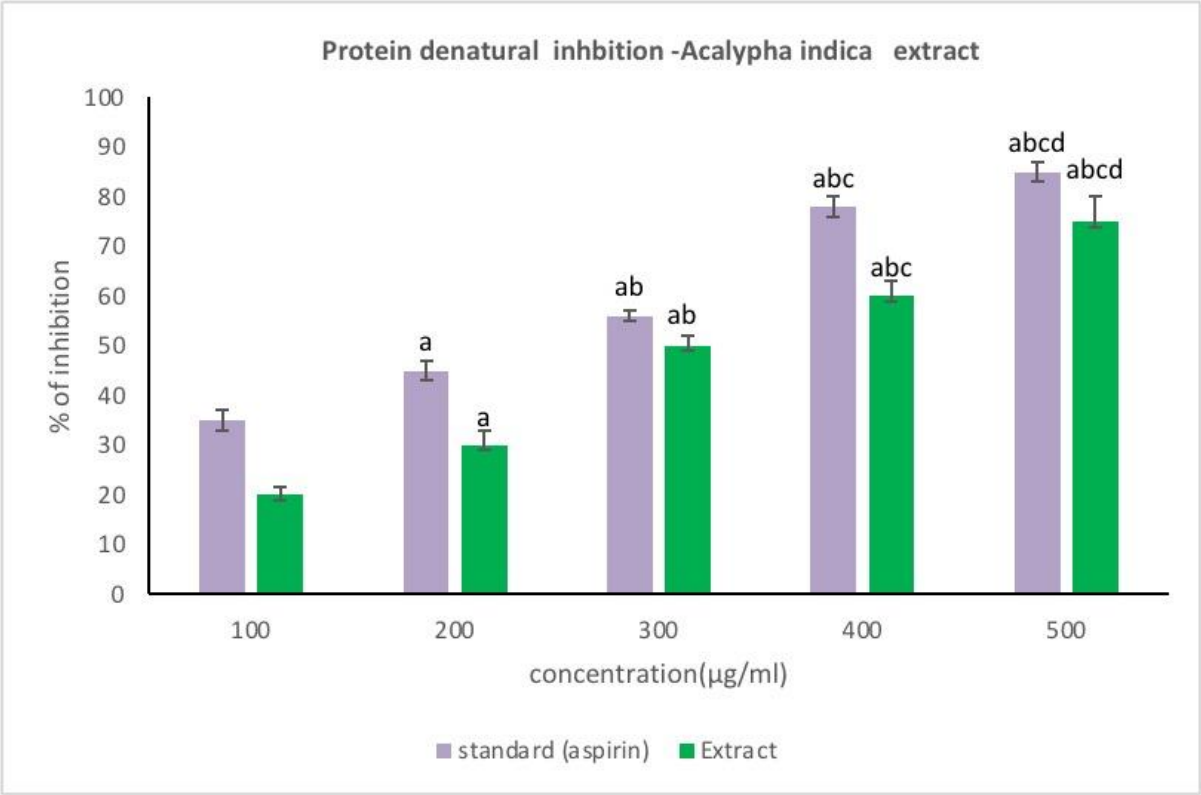

Figure 2 :- Bar graph depicts the Protein denaturation inhibitory activity of seed extract of *Acalypha indica* extract. Each bar represents the mean ± SD of 6 observations. The **x-axis** represents the concentration (micro g/ml) of the plant extract and the **y-axis** represents the % of inhibition of the plant extract. The purple bar represents the standard (aspirin) and the green bar represents the extract. Significance at the levels of $P < 0.05$. a-compared with 100 µg; b-compared with 200 µg; c-compared with 300 µg.; d-compared with 400µg.

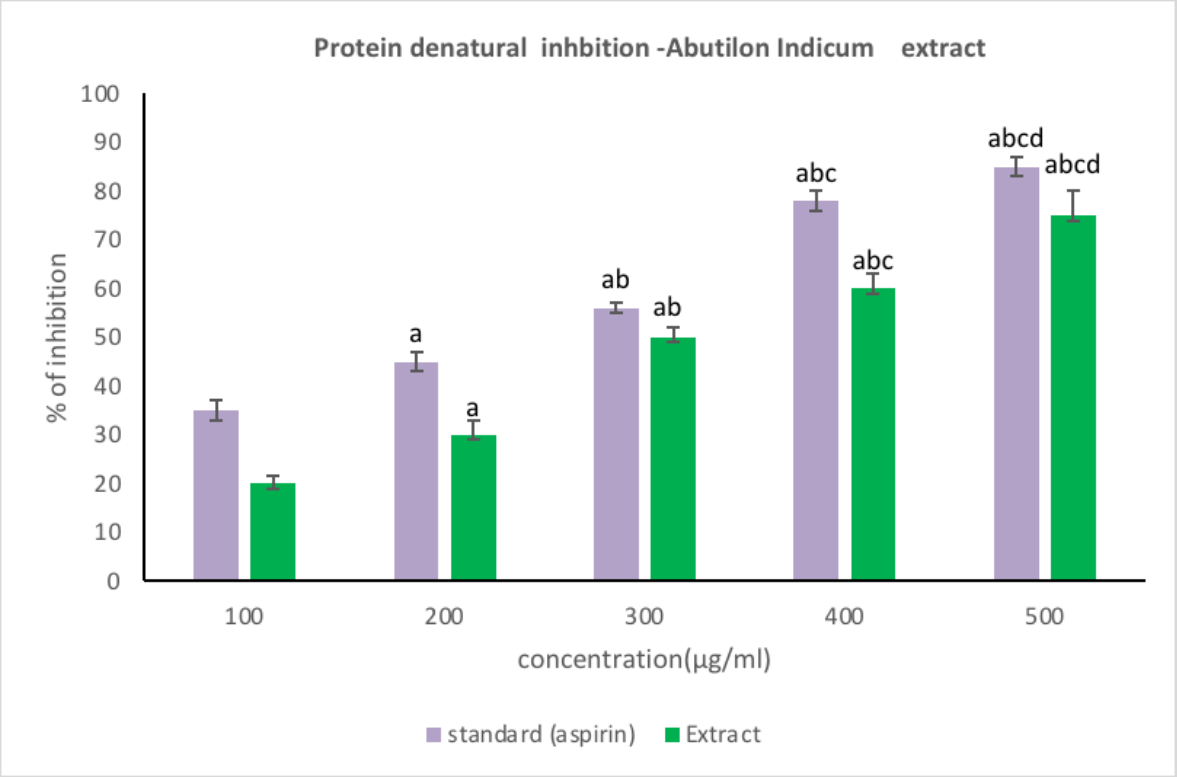

Figure 3 :- Bar graph represents the Protein denaturation inhibitory activity of seed extract of *Abutilon Indicum*. Each bar represents the mean ± SD of 6 observations. The **x-axis** represents the concentration (micro g/ml) of the plant extract and the **y-axis** represents the % of inhibition of the plant extract. The purple bar represents the standard (aspirin) and the green bar represents the extract. Significance at the levels of $P < 0.05$. a-compared with 100 µg; b-compared with 200 µg; c-compared with 300 µg.; d-compared with 400µg.

DISCUSSION:

Nature provides an enormous source of pharmacologically active molecules for new discovery of drugs (30). Many medicines of plant origin have been used for a long period of time to treat different kinds of disorders (31). Therefore it is essential that steps should be taken to introduce new medicinal plants and to develop cheaper, effective and safe analgesic and anti-inflammatory drugs so that patients can get access to much preferred mode of medication which is more safe and natural compared to synthetic drugs (32). Natural medicines are effective in action without or very minimal side-effects as a part of the investigation on the mechanism of the anti-inflammatory activity, the ability of plant extract to inhibit protein denaturation was also studied due to the medicinal properties of *Tecoma stans*, *Acalypha indica* and *Abutilon indicum* plant leaf extract (33). In the present investigation the plant leaf extract of *Tecoma stans, Acalypha indica and Abutilon indicus*

was tested for their anti-inflammatory activities (18). According to the WHO report, about 80% of the world population still rely mainly on natural and herbal remedies (19).

Pain and inflammation remains one of the major health problems among the population. Excessive inflammatory response has damaging effects, which can lead to multiple organ dysfunction syndrome and death (20). Novel potent analgesic and anti-inflammatory drugs without many side effects from nature are under evaluation (21). Nature is man's best friend (34). Natural medicines are effective in action without or very minimal side-effects as a part of the investigation on the mechanism of the anti-inflammatory activity, the ability of plant extract to inhibit protein denaturation was also studied due to the medicinal properties of *Tecoma stans*, *Acalypha indica* and *Abutilon indicus* plant leaf extract (33). In the present investigation the plant leaf extract of *Tecoma stans, Acalypha indica and Abutilon indicus* was tested for their anti-inflammatory activities. According to the WHO report, about 80% of the world population still rely mainly on natural and herbal remedies (22).

Tecoma stans (Bignoniaceae) is an ornamental plant found all over India. It is not a toxic plant and is used as a remedy for diabetes and for feeding cattle and goats in some countries (23). Leaves of *Tecoma stans* contain alkaloids, tecomine and tecostamine, which are potential hypoglycemic agents when provided intravenously (33,35). Anthranilic acid is responsible for the antidiabetic activity of this plant and their roots are powerful diuretic and vermifuge (36). The plant leaf extract of Acalypha indica also demonstrated antiinflammatory effect in a dose-dependent manner. From a study conducted by Rahman MA et al 2010, (7), it was observed that the maximum inhibition by the extract of this plant was at 250 mg/kg body weight after three hours of ingestion, this extract was then compared to that of the standard drug phenylbutazone at a dosage of 100 mg/kg body weight (23,24). The obtained results provide support for the use of this plant leaf extract in traditional and herbal medicine (25). *Abutilon indicum* is commonly called 'Country Mallow'. It belongs to the family Malvaceae. It is a perennial plant that can grow up to 3 m in height. Abutilon indicum is abundantly found as a weed in the sub-Himalayan tract and in the hotter regions of India (37). *Abutilon indicum* along with its other properties is also reported to have hypoglycemic, hepatoprotective, antimicrobial, male contraceptive, and antidiarrheal activities (8).

In clinical scenarios, there are much more proteins that are involved in inflammation. While indication is provided for potential protein protective activity, in vivo testing is necessary to prove the clinical utility of the extracted phytochemicals (26).

As discussed earlier, the synergistic action of plant leaf extracts like *Tecoma stans*, *Acalypha indica* and *Abutilon indicum* which was used in our study are worthy of investigation (27). But due to wide variations, it is essential to carry out the isolation of specific components by suitable means to identify their properties by carrying out the necessary tests. But from an economic perspective, the process can be disadvantageous due to destruction of plants for research purposes, however the isolation of flowers and leaves is less impactful than barks of trees (27,28).

In future, the comparisons should be made among leaves and flowers isolated in various seasons around the year. Research on various herbal formulations can create awareness and help mankind from various disorders. There is enormous scope for future research and further clinical and former logical investigation should be conducted to investigate the unexpected potential of these plants.

CONCLUSION:

In this study, the results indicate that the leaf extracts of the selected plants contain anti-inflammatory activity. The research revealed *Tecoma stans*, *Acalypha indica* and *Abutilon indicus*, their leaf extract are important medicinal plants with diverse pharmacological spectrum. They showed the same amount of inhibitory activity towards protein denaturation and hence possess the same level of anti inflammatory activity. Lots of studies have been carried out with extract of different parts of the plant. This particular study summarises some important pharmacological studies on anti-inflammatory activity. Hence this article effort has been taken to collect and compile the details on *Tecoma stans*, *Acalypha indica* and *Abutilon indicus* which will be useful to the society to venture into a field of alternate system of medicine.

NOTE:

The study highlights the efficacy of " herbal medicine " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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