**International Journal of Botany Studies**
**www.botanyjournals.com**
**ISSN: 2455-541X**
**Received: 24-12-2021, Accepted: 08-01-2022, Published: 25-01-2022**
**Volume 7, Issue 1, 2022, Page No. 491-497**

# *In vitro* anticancer effect of modified bentonite clay using matura tea tree

**Priya V, Ancy Devadas**
Research Scholars, Women's Christian College, Manonmaniam Sundaranar University, Thirunelveli, Tamil Nadu, India

**Abstract**
Medicinal plants are moving from margin to classic use with a more number of people looking for treatment and health approaches free from effects caused by pandemic diseases. The pharmacological properties and healing effects of Matura tea tree and its secondary metabolites investigations verify its importance as an appreciated healing plant. Currently ailments rise in an alarming rate and it is a must to synthesize new medicine inorder to kill the pathogenic bacteria and virus. Cancer is also a deadly carcinogenic disease, which has been very common in the society. So inorder to overcome these ailments we have to create new drugs. The aim of this study is to identify new inhibitory agents in an era when carcinogenic cells continue to encounter human healthiness and the availability of new anticancer compounds is limited. Our results indicated that organo modified bentonite using Matura tea tree can act as anticancer agents. Accepting the anticancer mechanism of organo modified bentonite using Matura tea tree can lead to their safe use or design of new antibiotic products.

**Keywords:** anticancer assay, bentonite clay, clay minerals, medicinal plant, matura tea tree

## Introduction
Clay is a type of fine-grained natural soil material containing clay minerals. Clays develop plasticity when wet, due to a molecular film of water surrounding the clay particles, but become hard, brittle and non–plastic upon drying or firing (Bergaya *et al*., 2006; Boggs and Sam, 2006; Boylu and Feridun, 2011) [2, 3, 4]. Most pure clay minerals are white or lightcoloured, but natural clays show a variety of colours from impurities, such as a reddish or brownish colour from small amounts of iron oxide (Breuer and Stephen, 2012; Churchman *et al*., 2006) [5, 6].
Clay minerals are the characteristics minerals on the earth found near planetary surface (the surface where the outer crust of the object comes in contact with atmosphere) environment with variable amount of ions like iron, magnesium, alkali metals, alkaline earth metals and other cations. They are considered as important constituents of soil and form by digenetic and hydrothermal alteration of rocks in presence of water. They are commonly found in fine grained sedimentary rocks such as shale, mudstone and siltstone. Clay minerals act as "chemical sponges" as they have capacity to hold water and dissolved plant nutrients eroded from other minerals due to the presence of some unbalanced electrical charge on their surface. As water is essential for clay minerals formation, therefore, most of the clay minerals are known as hydrous alumina silicate or hydrous aluminium phyllosilicate (Tong, 2000; Hillier, 2003) [28, 17].
The formation of clay minerals is due to the chemical weathering of rock. The chemical and structural composition of clay minerals is found to be similar to the primary minerals which originate from the crust of earth mainly from igneous or metamorphic rocks. Transformations may occur in ambient conditions. Although some of the most resistant primary minerals such as quartz, micas and feldspar may remain in soils whereas other less resistant primary minerals (pyroxenes, amphiboles) are susceptible to breakdown by weathering, thus forming secondary minerals. The resultant secondary minerals are the formed due to either modification of the primary mineral structure (incongruent reaction) or neoformation through precipitation or recrystallization of dissolved constituents of primary minerals into a more stable structure (congruent reaction). These secondary minerals are most probably defined as phyllosilicates because, as the name suggest (Greek: phyllon, leaf), they exhibit a platy or flaky structure with irregular edges; while one of their most important basic structural units is an extended $SiO_4$tetrahedra sheet. As the clay minerals are most important component of the soil, they are usually ultra-fined particles having less than 2 µm sized particles.
Clay minerals may be divided into four major groups; these include the kaolinite group, the montmorillonite/smectite group, illite group and the chlorite group. The kaolinite group has three members, including kaolinite, dickite, and nacrite, each with a formula of$Al_2Si_2O_5(OH)_4$. Montmorillonite, talc, pyrophyllite, Saponite, and nontronite are a few members of the larger smectiteclay group. The general formula for the chemical structure of this group is (Ca, Na, H) (Al, Mg, Fe, Zn)$_2$ (Si, Al)$_4O_{10}(OH)_2XH_2O$. The illite group is represented by the mineral, illite, the only common clay type. The general formula is (K,H) $Al_2$(Si, Al)$_4O_{10}(OH)_2XH_2O$ (Faheemuddin, 2008) [14].
Bentonite is one of the clay minerals that possess interlayer of silica and alumina. The formula for bentonite clay is $Al_2H_2O_{12}Si_4$. It is naturally formed from volcanic ash weathering in the presence of water. It can be used

without any modification or can be modified using certain physiochemical treatments based on their applications. It is mainly used in structural polymers, ceramic bodies, drilling fluids, and also in adsorption catalytic processes. The incorporation of any molecules within the nanoclay system does not affect the nature of the molecules. When it is modified by the addition of any plant alkaloids, it is known as organobentonite. This incorporation occurs by different molecular interactions such as hydrogen bonding, ion exchange, and dipole interaction (Anand and Jeslin, 2018) [1]. Bentonite is an aluminium phyllosilicate generated frequently from the alteration of volcanic ash, consisting predominantly of smectite minerals, mostly montmorillonite (James *et al*, 2008; Asad *et al*, 2013) [18, 10].

Structure of clay particles is perceived in layers where each layer is composed of two types of structural sheets: octahedral and tetrahedral. The layers present in MMT are composed of a 2:1 structure i.e. two tetrahedral silica sheets sandwiching a central octahedral alumina sheet. Due to an isomorphic substitution within the layers (e.g. $Al^{3+}$ for $Si^{4}$ in the tetrahedral sheet and $Fe^{2+}$ or $Mg^{2+}$ for $Al^{3+}$ in the octahedral sheet) the clay layers have negative crystal charge which is balanced by exchangeable cations such as $Na^{+}$, $K^{+}$, $Ca^{2+}$ in the interlayer together with water molecules bonded by ion-dipole forces. The hydration of these inorganic cations causes the clay mineral surface to be hydrophilic. (Xi *et al*., 2007; Banik *et al*., 2015; Rodriguez *et al*., 2015) [29, 7].

Medicinal plants are the "backbone" of traditional medicines (Farnswoth, 1994). The World Health Organization (WHO) reported that 4 billion people (80% of the world's population) use herbal medicines for some aspect of primary healthcare (Mukherjee, 2002; Bandaranayake, 2006; Bodeker *et al.*, 2005) [21, 8]. In rural areas of the developing countries they continue to be used as the primary source of medicine (Chitme *et al*., 2003) [12]. These medicinal plants are considered as a rich respurce of ingredients which can be used in synthesis and drug development. *Sennaauriculata* (L.)Roxbis a native plant found in India and belongs to family Fabaceae. Its synonym is *Cassia auriculata* L and commonly known by other names as avaramsenna, matura tea, styptic weed or tanner's cassia (Lim, 2013) [19]. In Tamil, it is called as avaram tree. It is distributed throughout hot deciduous forests of India. It is available in dry regions of Madhya Pradesh, Tamilnadu, Rajasthan and other parts of India (Guruprasad and Reddy, 2016) [16, 42]. The plant possessed antipyretic, (Rao and Vedavathy, 1991) [25] hepatoprotective, anti-peroxidative, anti-hyperglycemic (Pari and Latha, 2003) [23] and microbicide activity.

**Fig 1:** Matura tea tree

**Pharmacological activities of matura tea tree**

**Anti-inflammatory activity**

Methanolic extract of the Cassia species leaves was investigated against carrageenin, histamine, serotonin and dextran induced rat hind paw oedema. It exhibited significant anti-inflammatory activity against all these agents (Maitya *et al*., 1997).

**Antibacterial activity**

De-alcoholized extract of Cassia species seeds inhibited the growth of *Micrococcus pyogenesvar, Micrococcus citreus, Cornebacterium diphtheria, Bacillus megatherium, Salmonella typhosa, Salmonella paratyphi, Salmonella schottmuelleri* and *Excherichia coli* (Singh and Khan, 1990).

**Antifungal activity**

The leaf extract has shown the significant antifungal activity to inhibit the growth of *Candida albicans*, *Aspergillusniger*, *Sachharomycescerevisiae* and *Trichophytonmentagrophyte* (Lemli and Cuveela, 1975). It shows antifungal activity due to chrysophenol and crysophanic acie-9- anthrone and other authraquinones such as emodine, physcion and rhein (Mukharjee *et al.*, 1996; Acharya and Charrerjee, 1974).

**Antioxidant activity**

The methanolic extract of Cassia species seeds shows stronger antioxidant activity. It was found that it exhibits stronger antioxidant activity as compared to Alphatocopherol (Chakrabarty and Chawla, 1983). The phenolic active component, alaternin and non-rubrofusaringlucoside isolated from extract of Cassia species also showed a potent free radical scavenging activity.

**Applications of matura tea tree**

It is used as tonic, carminative and stimulant. Its leaves, seeds, and roots are used medicinally, primarily in Asia. It is believed to possess a laxative effect, as well as to be beneficial for the eyes. As a folk remedy, the seeds are often roasted, then boiled in water to produce a tea. Roasted seeds have also been used as a substitute for coffee. According to ayurveda the leaves and seeds are laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardio-tonic, expectorant, leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis (Shibata *et al.*, 1969).

## Materials and Methods

### Materials

Bentonite clay were obtained from Indian clays limited, Thiruvananthapuram. Matura tea tree were collected from our area.

### Methods

**Anticancer assay by MTT assay**

SKMEL (2500 cells/well) were seeded on 96 well plates and allowed to acclimatize to the culture conditions such as 37 °C and 5% $CO_2$ environment in the incubator for 24 h. The test samples were prepared in DMEM media (100 mg/mL) and filter sterilized using 0.2 µm Millipore syringe filter. The samples were further diluted in DMEM media and added to the wells containing cultured cells at final concentrations of 6.25, 12.5, 25, 50, 100 µg/mL respectively. Untreated wells were kept as control. All the experiments were done in triplicate and average values were taken in order to minimize errors. After treatment with the test samples the plates were further incubated for 24 h. After incubation period, the media from the wells were aspirated and discarded. 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells. The plates were further incubated for 2 h for the development of formazan crystals. The supernatant was removed and 100 µL DMSO (100%) were added per well. The absorbance at 570 nm was measured with micro plate reader. Two wells per plate without cells served as blank. All experiments were done in triplicates. The cell viability was expressed using the following formula:

$$Percentage\ of\ cell\ viability = \frac{Average\ absorbance\ of\ treated}{Average\ absorbance\ of\ control} \times 100$$

The $IC_{50}$ value is the half maximal inhibitory concentration of the sample. The $IC_{50}$ values were calculated using the equation for slope (y = mx + C) obtained by plotting the average absorbance of the different concentrations of the test sample (6.25-100 µg/mL) in Microsoft Excel.

## Results and Discussion

***In vitro* cytotoxic assay of modified bentonite clay using Matura tea tree**

The capability of cells were calculated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method. To study the cytotoxic effect of the modified bentonite clay using matura tea tree against normal hypotetraploid human cell line, MTT assay was performed.

**Table 1:** Morphology of the cell showing the effect of B-AS

| Sample Concentration (µg/mL) | Percentage Viability |
|---|---|
| Positive Control | 98.4345 |
| 6.25 | 94.69421 |
| 12.5 | 86.10774 |
| 25 | 73.22803 |
| 50 | 62.94046 |
| 100 | 48.80518 |

**B-AS –Bentonite clay using matura tea tree**

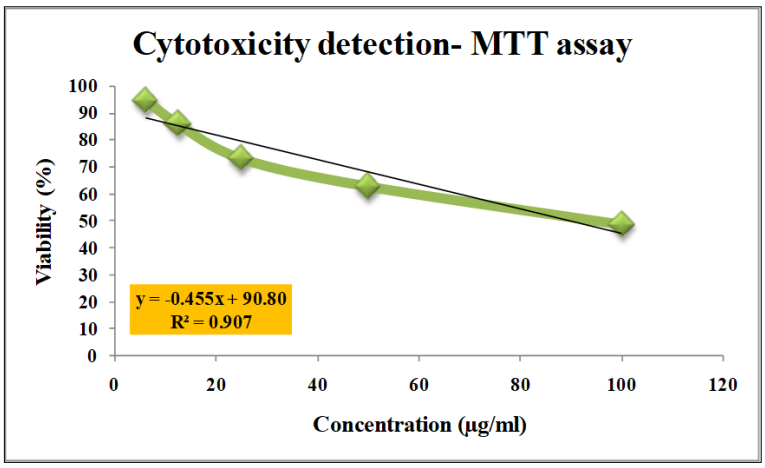


**Fig 1:** Cytotoxicity detection by MTT assay

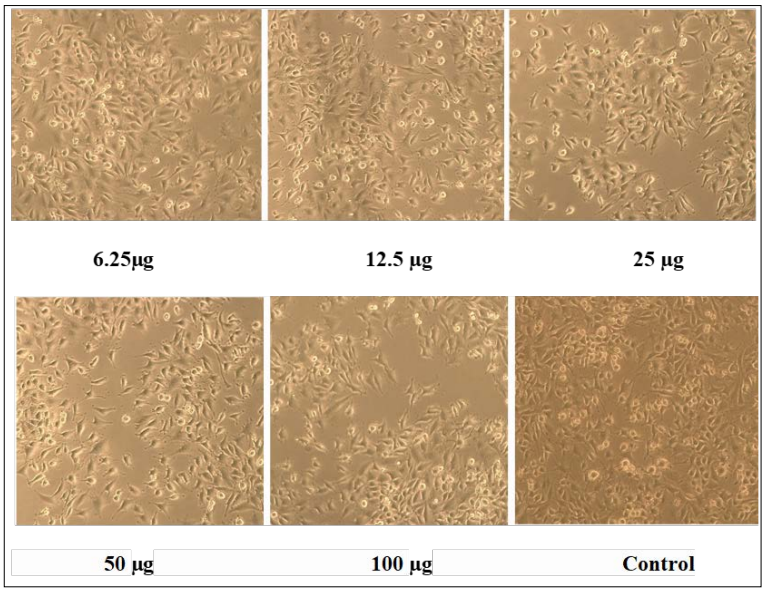


**Fig 2:** Morphology of the cell showing the effect of B-AS

From the above figure it was detected that no significant cytotoxic effect was observed in the normal hypotetraploid human cells cell line at the highest concentration (100ml) of the derivative that significantly affected the cancer cells. The morphology of the cell shows that the effect of bentonite clay using matura tea tree is moderately high when the concentration increases. Sample at various concentrations was added to check the cell capability. Dose dependent reduction in cell viability was observed in hypotetraploid human cells administered with different concentrations of the sample, B-AS**.** The IC 50 was observed with 89.67 µg/ml concentration of this sample. The modified clay using matura tea tree was taken for further anticancer effect on L6 cell line.

***In vitro* anticancer assay of modified bentonite clay using Matura tea tree**

The MTT *in vitro* cell proliferation assay is one of the most commonly used study for assessing initial anticancer activity of bentonite clay using matura tea tree. Stain is used to detect the chromatin condensation and decreasing cell viability appeared in a dose dependent and time dependent manner.

**Table 2:** Anticancer activity of BC-AS against L6 cell line

| Sample Concentration (µg/mL) | Percentage Viability |
|---|---|
| Positive | 99.59054 |
| 6.25 | 99.09008 |
| 12.5 | 98.77161 |
| 25 | 98.31665 |
| 50 | 98.13467 |
| 100 | 99.59054 |

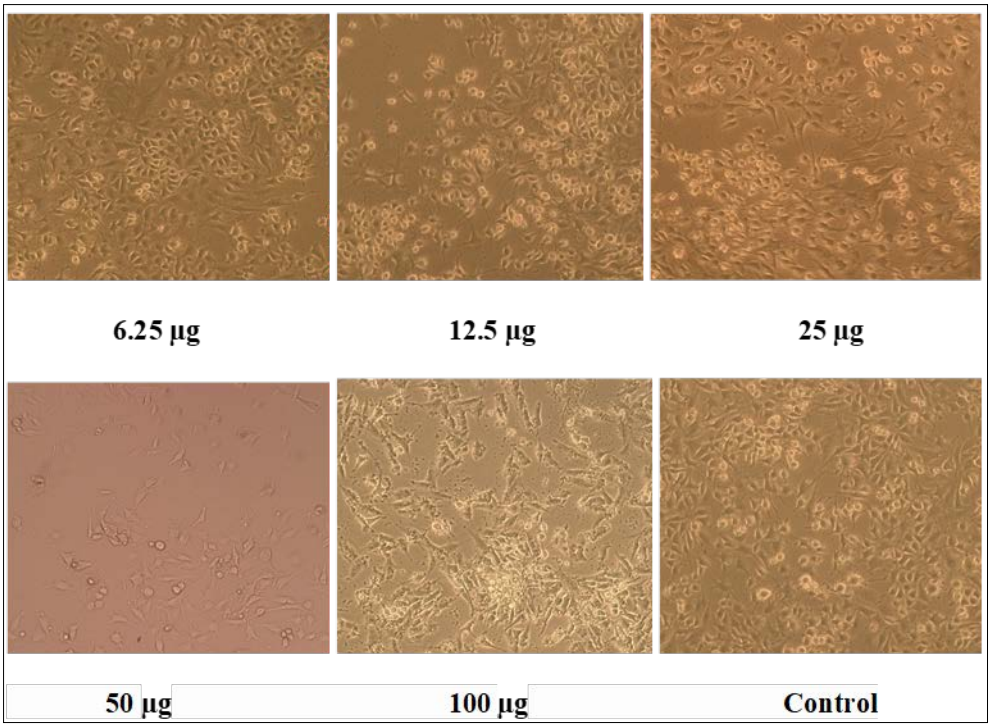


**Fig 3:** Morphology of the cell showing the effect of B-AS

In the present analysis, the colorimetric based assay is performed on a wide range of L6 rat myoblast cell lines. The modified bentonite clay using matura tea tree was the sample used to study the anti-proliferative potential against L6 cell line. The sample was found to cause no significant reduction in cell viability. This indicates that the sample is not cytotoxic to the normal cells.

**Conclusion**

The modified bentonite clay using Matura tea tree was subjected to anticancer assay because of its high antioxidant activity. The modified bentonite clay using Matura tea tree was found to cause no significant reduction in cell viability. This indicates that the clay mineral is not cytotoxic to the normal cells.

**References**

1. Anand Raj LFA, Jeslin J. 'Synthesis of bentonite nanoclay and incorporation of cassia fistula leaf extract to form organobentonite: characterization and its biomedical applications', Asian Journal of Pharmaceutical and clinical research,2018:11:9.
2. Bergaya F, Lagaly G. 'General introduction: Clays, clay minerals and clay science', Hand book of clay science,2006:5:1001-1009.
3. Boggs Sam. 'Principles of sedimentology and stratigraphy', Upper Saddle River, 2006.
4. Boylu, Feridun. 'Optimization of foundry sand characteristics of soda-activated calcium bentonite', Applied Clay Science,2011:52(1):104-108.
5. Breuer and Stephen, 2012,'The chemistry of pottery' Education in Chemistry,2012:17-20.
6. Churchman GJ, Gates WP, Theng BKG Yuan G. 'Chapter 11.1 Clays and Clay Minerals for Pollution Control', Handbook of Clay Science,2006:1:625-675.
7. Banik N, Jahan SA, Mostofa S, Kabir H, Sharmin N, Rahman M, et al. 'Synthesis and characterization of organoclay modified with cetylpyridinium Chloride' Journal of Science,2015:50:65-70.
8. Bandaranayake WM. 'Quality control, screening, toxicity, and regulation of herbal drugs, in Modern Phytomedicine', Turning Medicinal Plants intoDrugs, 2006.
9. Bodeker C, Bodeker G, Ong CK, Grundy CK, Burford G, Shein K. 'WHO Global Atlas of Traditional, Complementary and Alternative Medicine. Geneva, Switzerland' World HealthOrganization, 2005.
10. Asad A, Shantanu K, Mohammad AD, Raquibul H. 'Suitability of Bentonite Clay: An Analytical Approach', International Journal of Earth Science,2013:2:88-95.
11. Acharya TK, Chatterjee IB. 'Isolation of chrysophanic acid –9-anthrone, a fungicidal compound from Cassia species', Scientific Culture,1974:40:376.
12. Chitme HR, Chandra R, Kaushik S. 'Studies on anti-diarrheal activity of Calotropis gigantean in experimental animals', Journal of pharmacy and pharmaceutical sciences,2003:7:70-75.
13. Chakrabarty K, Chawla H. 'Terpenoids and phenolics from Cassia species stem bark', Indian Jornal of Chemistry,1983:22:1165-1166.

14. Faheemuddin. 'Clays, nanoclays, and montmorillonite minerals', Metallurgical and Materials transactions A,2008:39:2804-2814.
15. Farnswoth NR, Chinchester Wiley. 'Ethnopharmacology and drug discovery', Proceeding of Ciba Foundation Symposium,1994:185:42-59.
16. Guruprasad C Nille, Reddy KRC. 'A Phytopharmacological Review of Plant – Cassia auriculata', International Journal of Pharmaceutical and Biological Archives,2016:6:1-9.
17. Hillier SM, Coniglio L, Hardie F, Longstaffe J. 'Encyclopaedia of sediments and sedimentary rocks: Kluwer Academic', Clay Mineralogy,2003:47:139-142.
18. James OO, Adediran MM, Adekola FA, Odebunmi EO, Adekeye JID. 'Beneficiation and Characterisation of a Bentonite from North-Eastern Nigeria', Journal of the North Carolina Academy of Science,2008:124:154-158.
19. Lim TK. 'Sennaauriculata. In: flower. Edible medicinal and non-medicinal plants' Springer,2013:860-872.
20. Mukharjee PK, Saha K, Das J, Saha BP, Pal M. 'Antifungal activity of the leaf extract of Cassia species linn', Phytochemical Research Journal,1996:10:521-522.
21. Mukherjee PW. 'Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals', Business Horizons Publishers, 2002.
22. Maitya TK, Mandal SC, Mukherjee PK, Saha K, Dass J, Saha BP. 'Evaluation of hepatoprotective potential of Cassia species leaf extract', Natural Production of Science,1997:3:122.
23. Pari L, Latha M. 'Antihyperglycaemic effect of Cassia auriculata in experimental diabetes and its effects on key metabolic enzymes involved in carbohydrate metabolism', Journal of Physiology and Pharmacology,2003:30:38-43.
24. Rodrıguez FJ, Corte´s LA, Guarda A, Galotto MJ, Bruna JE. 'Characterization of cetylpyridinium bromide-modified montmorillonite incorporated cellulose acetate nanocomposite films', Journal of Material Science, 2015.
25. Rao KN, Vedavathy S.'Antipyretic activity of six indigenous medicinal plants of Tirmulahills', Journal of Ethnopharmacology,1991:33:193-196.
26. Singh VK, Khan AM. 'Medicinal Plants and Folklores - A Stratergy towards Conquest of Human Ailment's. Today and Tomorrow Printers and Publishers, 1990, 9.
27. Shibata S, Morishita E, Kaheda M, Kimura Y, Takido M, Takashashi S. Chemical journal of Pharmacy,1969:17:454.
28. Tong WK. 'Introduction to clayminerals', 2020.
29. Xi Y, Ray LF, Hongping H. 'Modification of the surfaces of Wyoming montmorillonite by the cationic surfactants alkyl trimethyl, dialkyl dimethyl, and trialkyl methyl ammonium bromides. Journal of Colloid and Interface Science',2007:30:150-158.
30. TresaSunitha George, Asha Krishnan K, Anjana RKE, George. 'Studies on Nano Kaolin Clay Reinforced PS-HDPE Nanocomposites', Indian Journal of Advance in Chemical Science,2013:1(4):202-206.
31. Tz N, Dintcheva G, Fillippone FP, Lo Mantia D, Acierno. 'Photo- oxidation behaviour of polyethylene/polyamide 6 blends filled with organo modified clay: Improvement of the photo-resistance through morphology modification', Polymer Degradation and Stability,2010:95:527-535.
32. Subramanian M, Goswami M, Chakraborty S, Jawali N. 'Resveratrol induced inhibition of *Escherichia coli* proceeds via membrane oxidation and independent of diffusible reactive oxygen species generation', Redox Biology,2014:2:865-872.
33. Sudhakar Chekuri, Lali Lingfa, Shivaprasad Panjala KC, Sai Bindu, Roja Rani Anupalli. 'AcalyphaindicaL. – an important medicinal plant: A Brief Review of its pharmacological properties and restorative potential', European Journal of Medicinal Plants,2020:31(11):1-10.
34. Sileola B Ogunlaja, Rajindar Pal. 'Effects of Bentonite Nanoclay and Cetyltrimethyl Ammonium Bromide Modified Bentonite Nanoclay on Phase Inversion of Water-in-Oil Emulsions', Colloids and Interfaces,2020:4(2):1-14.
35. Sinadinovic-Fiser S, Jankovic M, Borota O. 'Epoxidation of castor oil with peracetic acid formed in situ in the presence of an ion exchange resin', Chemical Engineering and Processing,2012:62:106-113.
36. Odom IE. 'Smectite clay minerals: properties and uses, Philosophical transactions of the Royal Society a: Mathematical', Physical and Engineering Sciences, 1984, 311.
37. Ogbemudia Joseph Ogbebor, Felix EbhodagheOkieimen David EhioqhilenOgbeifun, UzomaNdubuisiOkwu. 'Organomodified Kaolin as Filler for Natural Rubber', Chemical Engineering,2015:4(21):477-484.
38. Natalia Costa Dias, Patricia Arnssteiner, Maria Cristina Borba Braga. 'Characterization and Modification of a clay Mineral used in Adsorption Tests', Journal of Minerals and Materials Characterization and Engineering,2015:3:277-288
39. Nazile Ural. 'The importance of clay in geotechnical Engineering current topics in the utilization of clay in industrial and medical applications', 2018, 83-102.
40. Muhammad Fiayyaz, Khalid Mahmood Zia, Mohammad Zuber, TahirJamil, Muhammad Kaleen Khosa, Muhammad Asghar Jamal. 'Synthesisand characterization of polyurethane/bentonite nanoclay based nanocomposites using toluene diisocyanate', Korean Journal Chemistry Engineering,2013:31(4):644-649.

41. Hernández-Hernández KA, Illescas J, DíazNava MDC, Muro-Urista CR, Martínez, Gallegos S. 'Polymer-Clay Nanocomposites and Composites: Structures, Characteristics, and their Applications in the Removal of Organic Compounds of Environmental Interest', Medicinal chemistry,2016:6:201-210.
42. Guruprasad C Nille, Reddy KRC. 'A Phytopharmacological Review of Plant – Cassia auriculata', International Journal of Pharmaceutical and Biological Archives,2016:6:1-9.
43. Guruprasad C, Nille KRC, Reddy. 'A Phytophrmacological Review of plant – Cassia auriculata', International Journal of Pharmaceutical and Biological Archives,2015:6(6):1-9.
44. Eisenhour, DD, Brown RK. 'Bentonite and Its Impact on Modern Life', Elements,2009:5(2):83-88.
45. De Oliveira CIR, MCG Rocha, ALN Da Silva, Bertolino LC. 'Characterization of bentonite clays from Cubati, Oaraiba (Northeast of Brazil)', Ceramica,2016:62:272-277.
46. Ardhyanata H, Ismail H, Takeichi T, Judawisastra H. 'Preparation and properties of vinyl acetate effects of organo clay loading and methyl ethyl ketone', Polymer Plastic Technology,2006:45:1285.
47. Antonova, ES, Bernardy EE, Hammer BK. 'Natural competence in Vibrio cholerae is controlled by a nucleoside scavenging response that requires CytR- dependent antiactivation', Molecluar Microbiology,2012:5:1215-1231.
48. Alberto Mannu, Gina Vlahopoulou, Veronica Sireus, Giacomo Luigi Petretto, Gabriele Mulas, Sebastiano Garroni. 'Bentonite as a Refining Agent in Waste Cooking Oils Recycling: Flash Point, Density and Color Evaluation', Natural Product Communciations,2018:13(5):613-616.
49. AkinadeAkinwumi, AfolabiFunsho. 'Experimental Investigation of the use of local clay (Abbi, Delta State, Nigeria) as a substitute for foreign imported bentonite clay in the formulation of aqua base drilling Fluid', International Journal of Scientifics Engineering Research,2015:6(6):1138-1144.
50. Ailf Khan. 'Bleaching of Vegetable Oil using Organic Acid Activated Fuller's Earth (BentoniteClay)', Global Journal of Researches in Engineering,2015:15(2):1-7.